Additional file 1: Supplemental Methods, Figures S1-S4 and Table S2

"Shared functions of plant and mammalian StAR-related lipid transfer (START) domains in modulating transcription factor activity" by Kathrin Schrick *et* al.

Supplemental Methods

Flow cytometry

GFP levels in live yeast cells were quantified by flow cytometry as in [1]. Yeast cells transformed with GSV:yEGFP3 constructs were grown to exponential phase (OD_{600} of ~0.500) in selection media containing low-flow fluorescence yeast nitrogen base without riboflavin and folic acid [2]. GFP positive and negative controls were pUG35 and pNF-1, respectively. For each sample, 2 x 10^6 cells were washed in 0.5 ml PBS, resuspended in 0.1 ml PBS for sonication, and another 0.9 ml was added prior to sample processing. Flow cytometry was performed using a BD Biosciences FACSAria Flow Cytometer Cell Sorter. Illumination was with a 200 mW 488 nm argon laser. Emission was detected through a 530/30 nm filter (FL1-H filter). 500,000 particles (yeast cells) were gated per sample.

Supplemental References

1. Niedenthal RK, Riles L, Johnston M, Hegemann JH: **Green fluorescent protein as a marker for gene expression and subcellular localization in budding yeast**. *Yeast* 1996, **12**(8):773-786.
2. Sheff MA, Thorn KS: **Optimized cassettes for fluorescent protein tagging in Saccharomyces cerevisiae**. *Yeast* 2004, **21**(8):661-670.

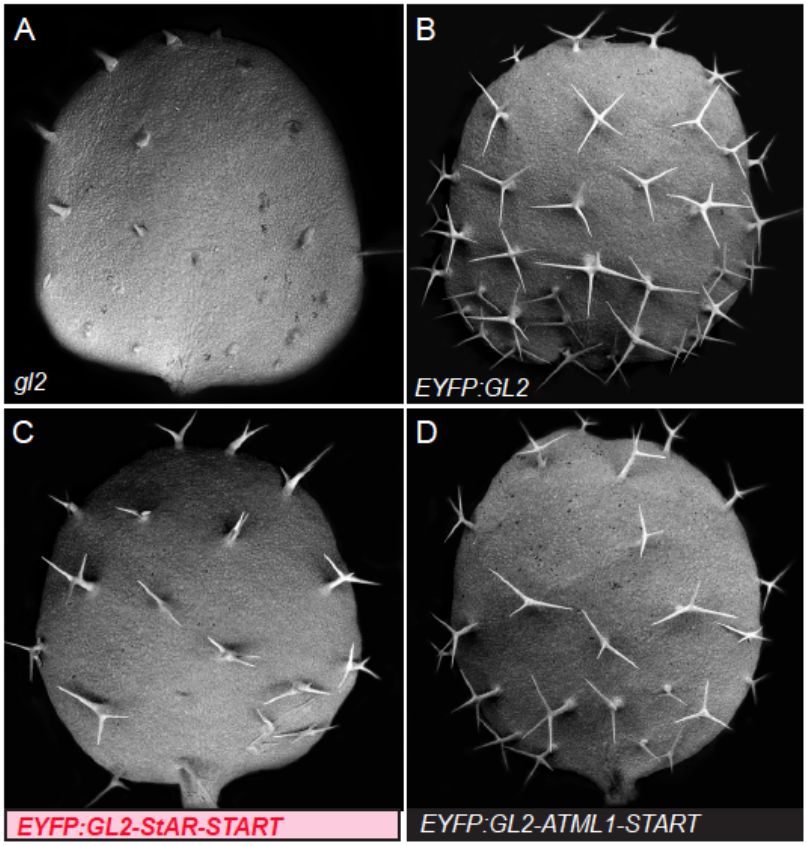

Figure S1. Trichomes on first leaves of *gl2* mutants transformed with *GL2* constructs. **(A-D)** Scanning electron micrographs (SEM) of first leaves. **(A)** *gl2* mutants exhibit a defect in differentiation of trichome cells as indicated by short unbranched trichomes that barely emerge from the epidermis. *gl2* mutants transformed with **(B)** *ProGL2:EYP:GL2* exhibit branched trichomes, indicating a rescue of the mutant phenotype, while *gl2* mutants transformed with **(C)** *ProGL2:EYFP:GL2-StAR-START* or **(D)** *EYFP:GL2-ATML1-START* display a partial rescue of the trichome differentiation defect.

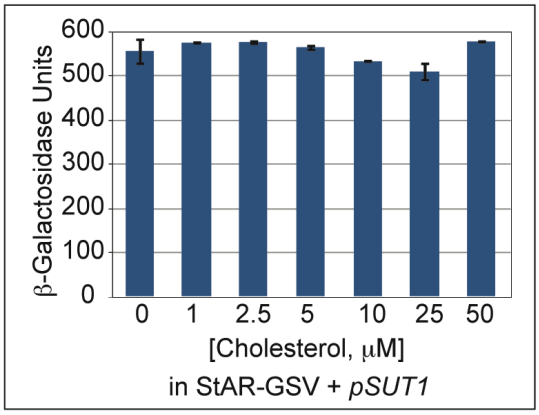

Figure S2. Exogenously supplied cholesterol does not alter activity levels of StAR-GSV. The addition of cholesterol in the range from 0-50 μM had no effect on the activity levels of yeast cells expressing the GSV construct containing the mouse StAR START domain together with the *pSUT1* plasmid. Error bars indicate standard deviations for two independent transformants in two trials.

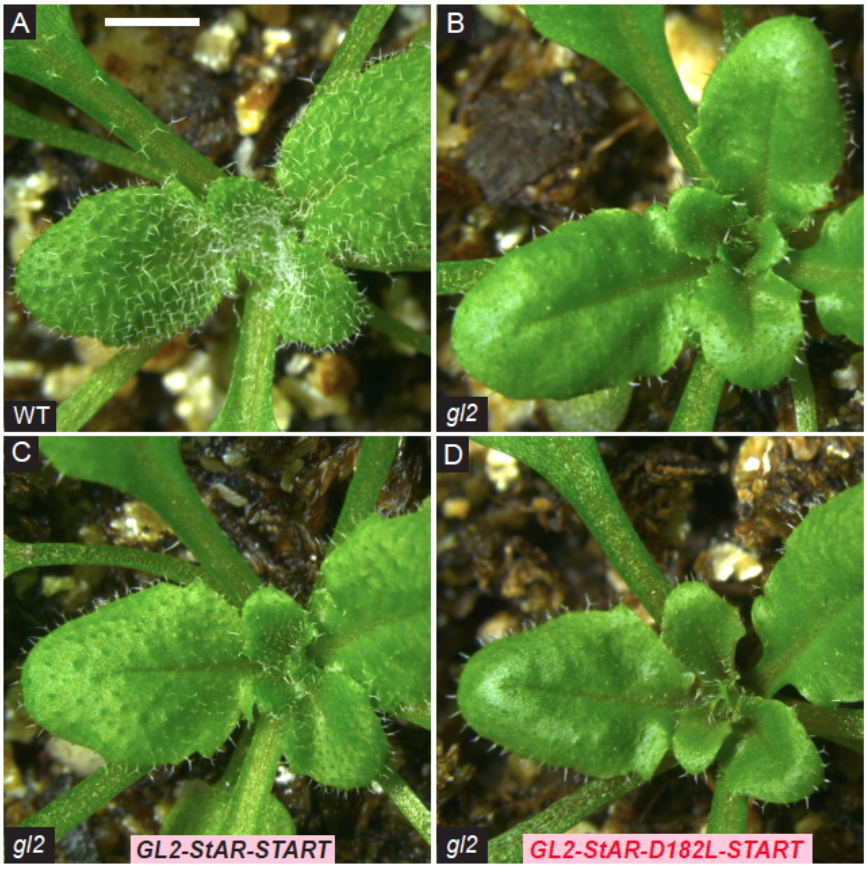

Figure S3. Rosette phenotypes of StAR-START versus the D182L missense mutant expressed in the GL2 transcription factor.

(A-D) Rosettes exhibiting leaf trichomes. **(A)** Wild-type (WT) level of trichomes in comparison to **(B)** *gl2* null mutant which displays a reduction in leaf trichomes.

(C-D) Representative *gl2* lines expressing **(C)** *ProGL2:EYFP:GL2-StAR-START* or **(D)** *ProGL2:EYFP:GL2-StAR-D182L-START*. While mouse StAR-START can partially replace the GL2-START domain, the missense mutation D182L results in a reduction in trichome cell differentiation. Scale bar = 2 mm. This figure is supplemental to Figure 4.

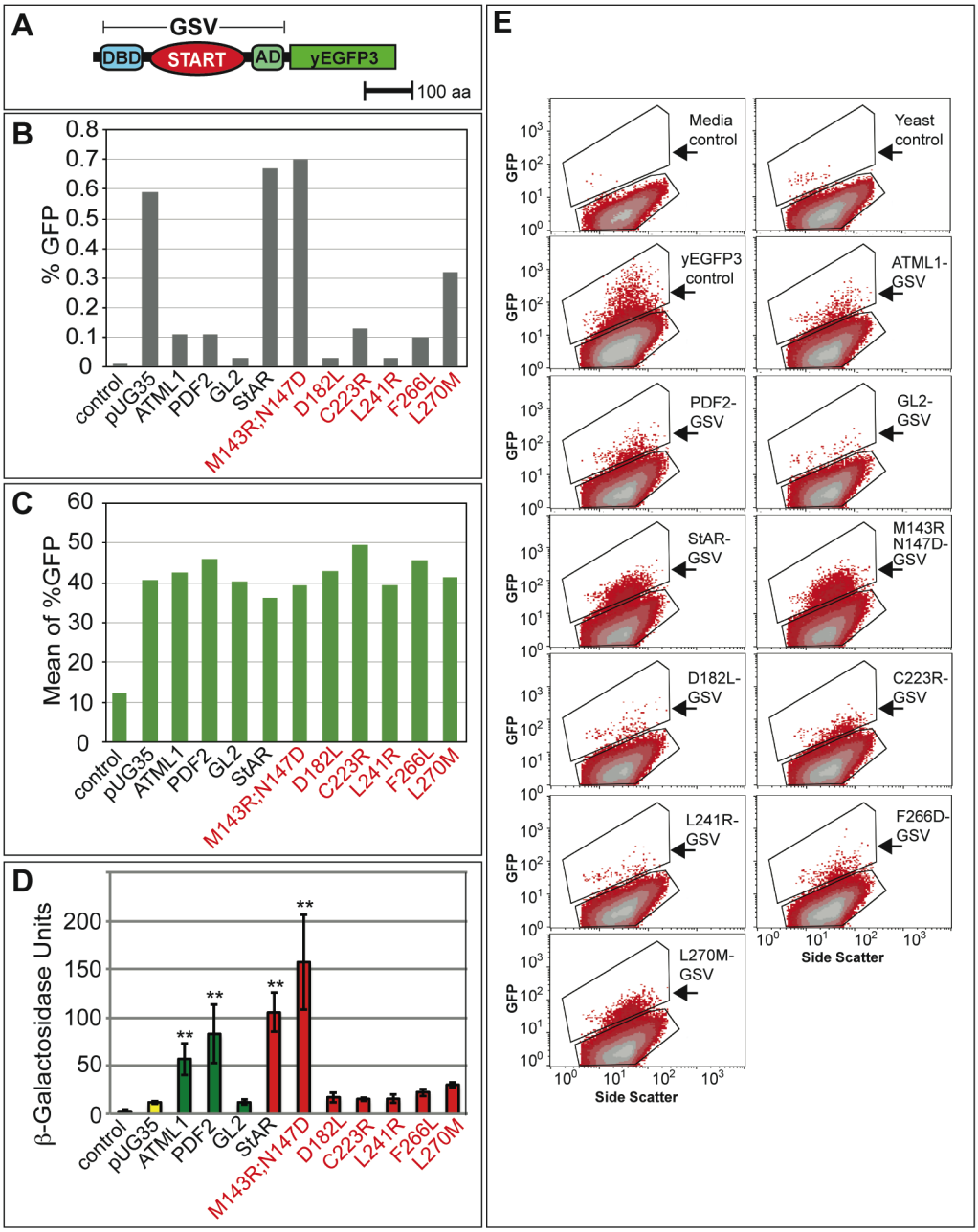

Figure S4. *In vivo* expression of GSV constructs as yEGFP3 fusions in yeast.

(A) Schematic of GSV translational fusion to yEGFP3.

(B) Flow cytometry data for the % GFP cells. The negative control which does not contain GFP corresponds to 0.01% GFP positive cells and the positive control which contains yEGFP3 alone (pUG35) corresponds to 0.59% GFP. The GFP-expressing cells exhibit % GFP values ranging from 0.03-0.70%.

(C) Mean values for % GFP from side scatter plots. The negative control shows a mean value of 12 while the positive control (pUG35) exhibits a mean value of 41. The GFP-expressing cells show mean values in the range from 36-47.
(D) Activity levels of the corresponding GSV-yEYFP3 constructs containing START domains from *Arabidopsis* ATML1, PDF2, and GL2 (green), and mammalian StAR and corresponding mutants (red) are indicated. Error bars show standard deviations for two independent transformants in three trials, and double asterisks indicate a significant increase in activity over the pUG35 control (Two-tiered *t*-test, $P \leq 0.05$).
(E) Flow cytometry side scatter plots of GFP positive yeast cells expressing yEGFP3. The top polygon from each plot indicates the population of cells that were gated as GFP positive (arrows). Side scatter is indicated on the X-axis and GFP signal is indicated on the Y-axis. "Media control" lacks yeast cells, while the "Yeast control" contains yeast cells that carry the same selectable marker (*URA3*) as the remaining samples albeit no GFP expression. The yEGFP3 control exhibits strong expression of yEGFP3 from the pUG35 plasmid. The sample order of the GSV-yEGFP3 constructs from top to bottom, right to left, corresponds to that in **A-D**. Each of the GSV samples indicates the presence of GFP positive cells in comparison to the negative controls.

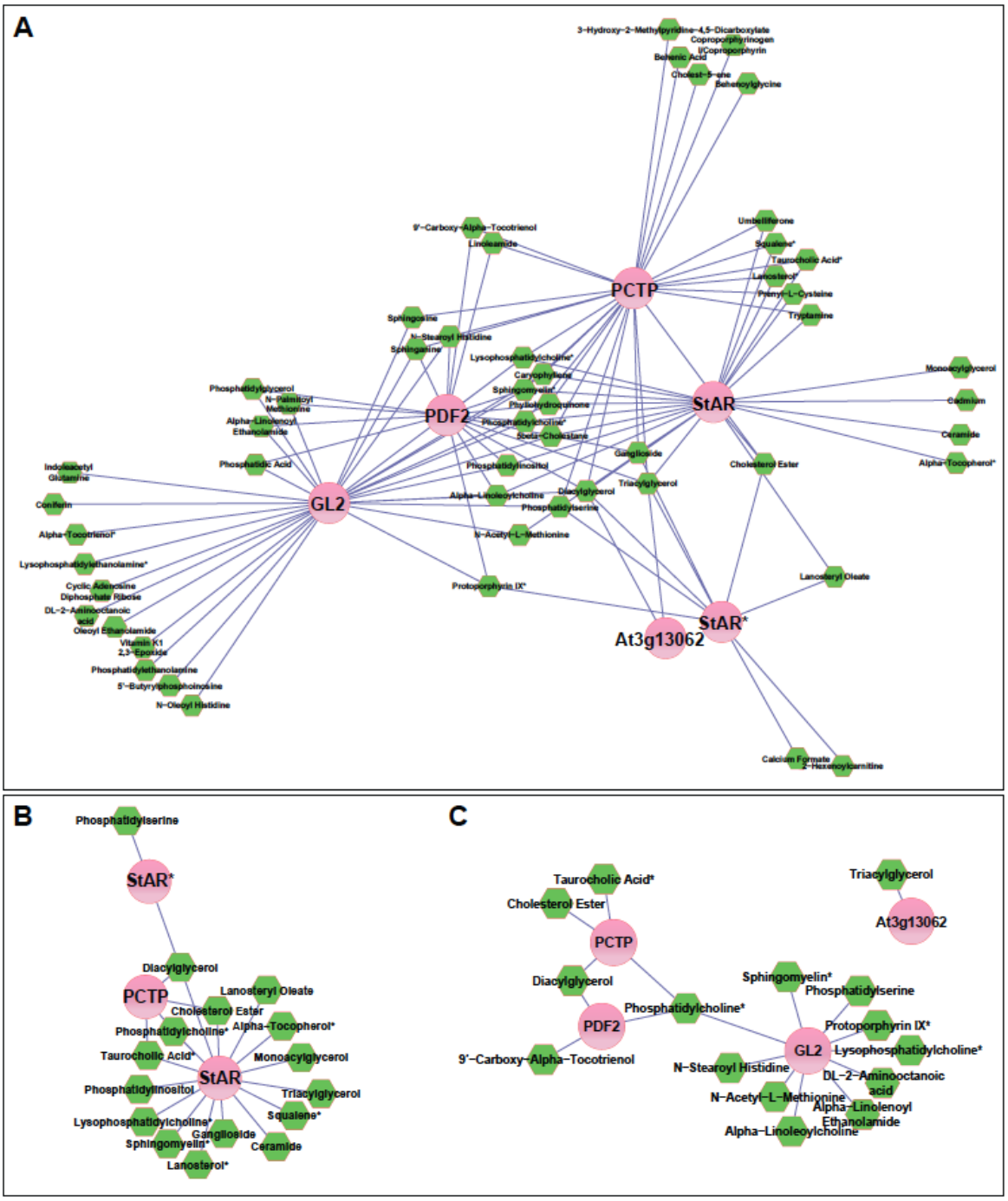

Figure S5. Protein-metabolite interaction network for mammalian and *Arabidopsis* START domains.

(A) Normalized protein-metabolite enrichment data expressed as the fold-change of domain-bound metabolite relative to the GV control greater than 4 were processed using Cytoscape to produce an edge-weighted interaction network in which larger elliptical nodes represent the different START domains tested and hexagonal nodes represent the interacting metabolites.

Distances between protein and metabolite nodes reflect the interaction strengths based upon the magnitude of fold-change – the shorter the edge the more enriched the metabolite.
(B) A sub-network was generated to compare and contrast the nature of protein-bound metabolites between the mammalian START domains, PCTP (human), StAR (mouse) and $StAR^{D182L}$* (mouse).
(C) A sub-network comparing the *Arabidopsis* and human PCTP START domains. The sub-networks **(B, C)** were filtered for interactions with a greater than 10-fold change in enrichment relative to the GV control and only high confidence metabolite assignments were included.
For all networks **(A-C)**, in cases where a node had multiple interactions with the same chemical sub-class of metabolite, e.g. PtCho, these interactions were combined and weighted to give one interaction. Metabolite names designated by asterisks were further validated by mass spectrometry, matching exact mass and retention time to a known standard analyzed under the same experimental conditions.

Table S2. Oligonucleotides used in this study. Nucleotide bases shown in bold denote restriction sites used for cloning or changed bases from site-directed mutagenesis unless otherwise indicated.

I. Primers for GL2 START domain deletion construct and GL2 START domain swaps. Homologous sequences for domain swap in-fusion cloning are indicated in bold.	
Name	**5'-3' sequence**
GL2_START_Δ_F	[Phos] GTC TTC TTC ATG GCT ACC AAC GTC CCC ACC
GL2_START_Δ_R	[Phos] GAG GGC AAA GAC GCC CGT GTA GAA ATC G
GL2_START_flank_right_F	**GTC TTC TTC ATG GCT** ACC AAC GTC
GL2_START flank_left_R	**GAG GGC AAA GAC GCC** CGT GTA
GL2_ATML1_START_F	**GGC GTC TTT GCC CTC** GAG GCT GAT AAG CCT ATG ATT G
GL2_ATML1_START_R	**AGC CAT GAA GAA GAC** GAG CCG CTC ACA TTG GCG GTC
GL2_EDR2_START_F	**GGC GTC TTT GCC CTC** AAC CAA GCA TTT TCC AGG AA
GL2_EDR2_START_R	**AGC CAT GAA GAA GAC** CCA CCC TTT TAG ATC AAT TTG
GL2_REV_START_F	**GGC GTC TTT GCC CTC** GAG GAG ACT TTG GCA GAG TTC
GL2_REV_START_R	**AGC CAT GAA GAA GAC** CCG CAA CGC GGA AAT GGT CA
GL2_mStAR_START_F	**GGC GTC TTT GCC CTC** GAC CAG GAG CTG TCC TAC ATC C
GL2_mStAR_START_R	**AGC CAT GAA GAA GAC** GCT GGC TTC CAG GCG CTT GC

II. Gene specific primers for PCR amplification and cloning of START domain coding regions in GSV plasmids.	
Name	**5'-3' sequence**
At1g64720_for_KpnI_218	CTCACCACGTTAACCCC**GGTACC**TCTTCCAAAGAG
At1g64720_rev_SacI_945	GTGAGCCATTATGGC**GAGCTC**GGATAAACCTGCTC
At2g28320_for_KpnI_418	TTGAGTAGCTCA**GGTACC**GACCATCACTCAAACTC
At2g28320_rev_SacI_1151	CTTGCACTTCTTG**GAGCTC**CCCCTGACGACAG
At3g13062_for_KpnI_201	CTCGGTTTCTCAATCT**GGTACC**TCCCAATCAGG
At3g13062_rev_SacI_934	AGCTTACAGC**GAGCTC**TGTGGGCCCTTGGGGTCG
At4g14500_for_KpnI_365	TGGCCTCAAGA**GGTACC**GATAACGGG
At4g14500_rev_SacI_1084	CCATTTGGGC**GAGCTC**AGATAGAGATGAGTCTG
At5g07260_for_KpnI_229	CTATATCCC**GGTACC**GCTACGTCTTTGACTG
At5g07260_rev_SacI_952	CTGGTCGAATAT**GAGCTC**ATTGTGACCAATTGAAGG
At5g35180_for_KpnI_634	CAAGGTCCA**GGTACC**CTTTTGAGGCAATCATC
At5g35180_rev_SacI_1382	TGGAACTTGGA**GAGCTC**AACCGTGGCGGAAG
At5g45560_for_KpnI_487	AGGACAACTATT**GGTACC**GGCCCTCCAGAATC
At5g45560_rev_SacI_1229	GATGCCATATT**GAGCTC**AACAGGGATCCTGATCGG
At5g54170_for_KpnI_344	TTTCAAGA**GGTACC**AAAACAAAGGAGAGATTGCC
At5g54170_rev_SacI_1065	CATGAAAGCAGACCG**GAGCTC**CTTGGTTCCTCC
ANL2 outer F_824	CCTCCTTAGAACTCGCTGTCGGCACC
ANL2 outer R_1780	GCTTGCTCCAATTGTGGACCGACG
ANL2_for_KpnI_915	GCAGCAGCAGCAGTCG**GGTACC**ATTAATGGG
ANL2_rev_SacI_1685	GGCGTTATTGATGT**GAGCTC**GTGAGATGTAACGG
ATHB8_for_KpnI_429	GACCCCT**GGTACC**CAGCCTCGTGATGC
ATHB8_rev_SacI_	GCCGCTGGTCT**GAGCTC**CCAACCTG
ATML1_for_KpnI_	ACATTTTGAGGTCG**GGTACC**ATACCTTCTGAGGC
ATML1_rev_SacI_	CAGGACTCGTTATCACGGA**GAGCTC**ACAAGC
CNA_for_KpnI_432	GGCATCT**GGTACC**CCTCAGAGAGATGC
CNA_rev_SacI_1162	GACGCCGTCC**GAGCTC**ATTAACACTAC
FWA outer F_450	GGCTGAGAATGCTAACTTGGAGCGGG
FWA outer R_1440	GCCACTTGTCCACCGAAGGACTCG
FWA_for_KpnI_592	GATTTTAGTGGT**GGTACC**AGGACGTCTGAGAAGG
FWA_rev_SacI_1361	GCAGACAATCC**GAGCTC**AATTTCAGTCAAGTTG
GL2_for_KpnI_A_	GTCTC**GGTACC**CTCGATTTCTACACGGGCGTC

GL2_rev_ SacI_A_	CTTTGGT**GAGCTC**GTTGGTAGCCATGAAGAAGAC
GL2_for_ KpnI_B_	TCGGCTCTCTC**GGTACC**TACACGGGCGTC
GL2_rev_ SacI_B_	TGTAACTCC**GAGCTC**GTCTTTGGTGGGGACG
GL2_for_KpnI_728	TCTACACG**GGTACC**TTTGCCCTCGAGAAGTCCCG
GL2_rev_SacI_1500	TCCGAGAGA**GAGCTC**GGTGGGGACGTTGGTAG
HDG1_for_KpnI_910	CAACCG**GGTACC**GTTAGTGATTTTGATC
HDG1_rev_SacI_1674	GCAGTTTATAGGGGATGG**GAGCTC**GGAAGTGG
HDG2_for_deltaSacI_759	CGTGGCTGCAATGGAAGA**A**CTCATGAGGATGGT
HDG2_rev_deltaSacI_791	ACCATCCTCATGAG**T**TCTTCCATTGCAGCCACG
HDG2_for_deltaSacI_1038	AGGAAACTATAATGGAGC**C**CTTCAAGTGATGAGTGC
HDG2_rev_deltaSacI_1073	GCACTCATCACTTGAAG**G**GCTCCATTATAGTTTCCT
HDG2_for_KpnI_712	ATCACTGCA**GGTACC**GAATCTGACAAACC
HDG2_rev_SacI_1415	GTAGCCATGAC**GAGCTC**TAACCGCTCGC
HDG3 outer F (625-650)	CATCCCCGTGTGTCTCCTCCTAATCC
HDG3 outer R (1511-1537)	TGGTCATTCCAGCAAAGAAGGTTCTCG
HDG3_for_KpnI	CCACTCGAGGGAAACC**GGTACC**CCTGCAGATGC
HDG3_rev_SacI	TCTTTCCATGGTTAGTTAGCGC**GAGCTC**GACAG
HDG4 outer F (539-562)	CTTGTGGCCACAATCTCCGCCTCG
HDG4 outer R (1447-1475)	TGTGACAGCTTCATCAAGTTCTTCCTCGC
HDG4_for_ KpnI	AAGAACAACAACGAT**GGTACC**TTGATTGCGG
HDG4_rev_SacI	AGGTAT**GAGCTC**AAGGTCAGTGATGTTTGTAGC
HDG5 outer F (808-836)	GACATGAGTGTATACGCTGGGAACTTTCC
HDG5 outer R (1766-1791)	GGTCCAAGACTGTCCATATGCAGTGC
HDG5_for_KpnI	CAACAAC**GGTACC**TTACTTGCGGATGAAGAAAAGG
HDG5_rev_ SacI	GCAGATGAAATTAC**GAGCTC**ATCAGTTATGTTTCTAGC
HDG8_for_deltaSacI_649	AGTGCGGTTGAAGAGCT**G**AAGCGGCTGTTTTTGGC
HDG8_rev_deltaSacI_683	GCCAAAAACAGCCGCTT**C**AGCTCTTCAACCGCACT
HDG8_for_KpnI_597	ACCACGACCA**GGTACC**GAAACGGATATGAGCC
HDG8_rev_SacI_1322	ATGGAGGA**GAGCTC**CATCCTCTCACAC
HDG9_outer_F_571	TTCTAACCGTCTCCCCGAGCCTTCAAGC
HDG9_outer_R_1547	GACTGTGGCGAGAAGTCGAGTTTGTTAACC
HDG9_deltaSacI_F_1329	CTTTGGCTACGGAGC**C**CGACGTTGGACCG
HDG9_deltaSacI_R_1357	CGGTCCAACGTCG**G**GCTCCGTAGCCAAAG
HDG9_for_KpnI_669	GGAAATGCAGAAT**GGTACC**CCACTATCTCAACTGG
HDG9_rev_SacI_1437	AACTCCGGGATT**GAGCTC**GTTGGGCAAGG**C**
HDG11_for_deltaKpnI_1000	CAGGAATGGGAGGTAC**G**CATGAGGGTGC
HDG11_rev_deltaKpnI_1028	GCACCCTCATG**C**GTACCTCCCATTCCTG
HDG11_for_KpnI_663	GCCTAACTTGGCT**GGTACC**GACATGGATAAGCC
HDG11_rev_SacI_1400	GAAGACGCTGGTACGGATAG**GAGCTC**AAATCTTTCACAC
HDG12_for_ KpnI_592	CCATCTCAGCCA**GGTACC**GTTTTATCAGAGATGG
HDG12_rev SacI_1361	ACTCCTCC**GAGCTC**AAGGGATGATG
MLN64_deltaSacI_F_867	GCCCTGTCCTGCGGAGCT**T**GTGTACCAGG
MLN64_deltaSacI_R_867	CCTGGTACAC**A**AGCTCCGCAGGACAGGGC
MLN64_for_ KpnI_	TCCTTTGCA**GGTACC**GACAATGAATCAGATGAAGAAG
MLN64_rev_ SacI_	TATCA**GAGCTC**CGCCCGGGCCCCC
PCTP_for_ KpnI_	GACTGC**GGTACC**ATGGAGCTGGCCGCCG
PCTP_rev_ SacI_	TCAACCCATGGATGCAATGTTCC**GAGCTC**TCTTTCATAGG
PDF2_for_KpnI	TTGAGGTCA**GGTACC**ATTCCTTCTGAGACTG
PDF2_rev_SacI	TATCACGGA**GAGCTC**ACCAGGAATGTTGC
PHB_for_ KpnI_463	AACCCAAATCCTCAG**GGTACC**CAACGTGATGC
PHB_rev_ SacI_1198	CAGGTTG**GAGCTC**TCCACCATACTG
REV_for_KpnI_423	GGTCACAACTCCTCAG**GGTACC**CTTAGAGATG
REV_rev_SacI_1162	CAGCAGGCTG**GAGCTC**TAATCCATACACTACT

mStAR_for_ KpnI_	GTCAGTCCTT**GGTACC**CAACTGGAAGCAACACTC
mStAR_rev_ SacI_	TTAACACTG**GAGCTC**AGAGGCAGGGCTGGC

III. Primers for sequencing plasmid inserts, construction of GV plasmid, or cloning of the yEGFP3 expression vector (pUG35) and protein expression vector BG1805

Name	5'-3' sequence
GSV_seq_for	TCCCAAAACCAAAAGGTCTCCGCTG
GSV_seq_rev	CCCCAACATGTCCAGATCGAAATCG
Gal4DBD_for_1	ATGAAGCTACTGTCTTCTATCGAAC
Gal4DBD_rev_276	CAATGCTTTTATATCCTGTAAAGAATCC
Gal4_NruI_for_282	TACCCCTGCAGCTGCG**TCGCGA**CTAGAGGATCC
Gal4_NruI_rev_314	GGATCCTCTAG**TCGCGA**CGCAGCTGCAGGGGTA
VP16_NruI_for_1182	TGCGGGCTCTACTTCATCG**TCGCGA**CACTTAGACGGCG
VP16_NruI_rev_1219	CGCCGTCTAAGTG**TCGCGA**CGATGAAGTAGAGCCCGCA
pUG35_seq_3117R_MET25p	TTCCTTCGTGTAATACAGGGTCG
pUG35_seq_2964F_yEGFP	ACCAAAATTGGGACAACACCAGTG
pUG35_MET25p_for_207	GCACCTTGTCCAATTGAACACGC
pUG35_yEGFP_rev_730	ACCTTCTGGCATGGCAGACTTG
pUG35_for_ATG	CATCCATACTCTAGA**ATG**AGTGGATCCCCCGGGC
pUG35_rev_ATG	GCCCGGGGGATCCACT**CAT**TCTAGAGTATGGATG
pGSV_for_BamHI	AAGCAA**GGATCC**TGAAAGATGAAGCTACTGTC
pGSV_rev_EcoRI	TCGCGC**GAATTC**CCCACCGTACTCG
pGS_rev_EcoRI	ACTATAGGGC**GAATTC**GAGCTCCACC
pG_rev_EcoRI	GTCTAAGTG**GAATTC**GGTACCTAACAATGC
GSV_for_pENTR TOPO	CACCATGAAGCTACTGTCTTCTATCGAAC
GSV_rev_pENTR TOPO	TGCCCCACCGTACTCGTCAATTCCAAG

IV. Primers for site-directed mutagenesis of mouse StAR START domain

Name	5'-3' sequence
StAR M143R;N147D_for (atg->agg;aac->gac)	GC ATG GAG GCC A**G**G GGA GAG TGG **G**AC CCA AAT GTC
StAR M143R;N147D_rev	GAC ATT TGG GT**C** CCA CTC TCC C**C**T GGC CTC CAT GC
StAR R181L;D182L_for (cga->cta;gac->ctc)	CTG GTG GGG CCT C**T**A **CT**C TTC GTG AGC GTG CGC
StAR R181L;D182L_rev	GCG CAC GCT CAC GAA G**AG** TA**G** AGG CCC CAC CAG
StAR R181L_for (cga->cta)	G GGG CCT C**T**A GAC TTC GTG AGC GTG CG
StAR R181L_rev	CG CAC GCT CAC GAA GTC TA**G** AGG CCC C
StAR D182L_for (gac->ctc)	CTG GTG GGG CCT CGA **CT**C TTC GTG AGC GTG CGC
StAR D182L_rev	GCG CAC GCT CAC GAA G**AG** TCG AGG CCC CAC CAG
StAR C224R_for (tgc->cgc)	GAA CAC GGC CCC ACC **C**GC ATG GTG CTT CAT CC
StAR C224R_rev	GG ATG AAG CAC CAT GC**G** GGT GGG GCC GTG TTC
StAR L241R_for (ctg->cgg)	CC AAG ACT AAA CTC ACT TGG C**G**G CTC AGT ATT GAC C
StAR L241R_rev	G GTC AAT ACT GAG C**C**G CCA AGT GAG TTT AGT CTT GG
StAR F266D_for (ttc->gac)	CC TA TCG CAG ACC CAG ATA GAG **GA**C GCC AAC CAC C
StAR F266D_rev	G GTG GTT GGC G**TC** CTC TAT CTG GGT CTG CGA TA GG
StAR L270M_for (ctg->atg)	GAG TTC GCC AAC CAC **A**TG CGC AAG CGC CTG G
StAR L270M_rev	C CAG GCG CTT GCG CA**T** GTG GTT GGC GAA CTC