

Table 1. GRNs built in this study.

net_type	study	note	sample_size	reference
tissue	stelpflug2016	B73	93	Stelpflug et al. 2016
	walley2016	B73	23	Walley et al. 2016
	zhou2018	B73	23	Zhou et al. 2018
		Mo17	23	
		BxM	23	
	leaf dev	leaf	40	Leaf Dev
	seed dev	embryo_endpsperm	61	Seed Dev
	dev atlas	combined	139	Dev Atlas
genotype	eitchen2013	seedling_leaf3	62	Eitchen et al. 2013
	hirsch2014	seedling	503	Hirsch et al. 2014
	leiboff2015	SAM	383	Leiboff et al. 2015
	fu2013	kernel	368	Fu et al. 2013
	lin2017	ear	26	Lin et al. 2017
		root	27	
		shoot	27	
		tassel	26	
		SAM	27	
	kremling2018	GRoot	274	Kremling et al. 2018
		GShoot	280	
		Kern	231	
		L3Base	264	
		L3Tip	266	
		LMAD	204	
		LMAN	262	
	shaefer2018	root_GCN	48	Shaefer et al. 2018
	huang2018	leaf	394	Huang et al. 2018
		root	176	
		SAM	406	
		seed	159	
	mazaheri2019	seedling	453	Mazaheri et al. 2019
	kaeppler2018	endosperm	89	Kaeppler et al. 2018
		internode	43	
		leaf	50	
		root	49	
		seedling	216	
	li2019	endosperm	121	Li et al. 2019
		internode	77	
		leaf	84	
		root	84	
		seedling	84	
tissue*genotype	lin2017	5*27	133	Lin et al. 2017
	kremling2018	7*306	1781	Kremling et al. 2018
	zhou2018	23*3	73	Zhou et al. 2018
	kaeppler2018	5*96	447	Kaeppler et al. 2018
	li2019	5*121	450	Li et al. 2019
	root dev	3*15	50	Root Dev
ril	li2013	SAM	107	Li et al. 2013
	wang2018	seedling_v2	617	Wang et al. 2018

Table 2. TF knockout mutant RNA-Seq datasets used in this study.

TF alias	TF name	TF ID	Study	Accession	Tissue	N
kn1	knotted1	Zm00001d033859	bolduc2012	PRJNA168086	meristem	10
cle7	clavata3/esr-related7	GRMZM2G372364	NA	PRJNA494874	ear tip	4
rbm48	RNA binding motif protein 48	Zm00001d054077	NA	PRJNA485828	endosperm	16
dek42	RNA binding motif protein 48	Zm00001d054077	NA	PRJNA485820	kernel	6
mads47	MADS-transcription factor 47	Zm00001d046053	NA	PRJNA289143	kernel	2
ufo1	unstable factor for orange1	Zm00001d000009	wittmeyer2018	PRJNA483200	pericarp	27
rel2	ramosa1 enhancer locus2	Zm00001d024523	liu2018	PRJNA475209	tassel	6
o2	opaque2	Zm00001d018971	zhan2018	PRJNA471036	endosperm	6
o2_b	opaque2	Zm00001d018971	li2015	PRJNA262503	endosperm	2
bzip22	bZIP-transcription factor 22	Zm00001d021191	li2018	PRJNA449867	kernel	6
fl3	floury3	Zm00001d009292	li2017	PRJNA375801	endosperm	6
fea4	fasciated ear4	Zm00001d037317	pautler2015	PRJNA262781	ear primordia	6
liguleless	liguleless1	Zm00001d002005	johnston2014	PRJNA260793	leaf primordia	24
nkd	naked endosperm1	Zm00001d002654	gontarek2016	PRJNA260183	aleurone, endosperm	2
sk1	silkless ears1	Zm00001d002970	NA	PRJNA483310	young V9 tassel	6
dek15	defective kernel15	Zm00001d052197	NA	PRJNA494076	kernel	6
ra1	RAMOSA1	Zm00001d020430	Eveland2014	PRJNA219746	ear	10
ra2	RAMOSA2	Zm00001d039694	Eveland2014	PRJNA219746	ear	10
ra3	RAMOSA3	Zm00001d022193	Eveland2014	PRJNA219746	ear	10
P1	Pericarp Color1	Zm00001d028842	Morohashi2012	PRJNA167802	pericarp	6
hda101	RPD3 histone deacetylase homolog	Zm00001d053595	Yang2016	PRJNA305809	seed	6

Table 3. GRN-predicted TFs supported by trans-eQTL hotspots.

ID	#_Targets	Supported eQTL study	Annotation
Zm00001d010048	17	li2013,liu2017,wang2018	Dehydration-responsive element-binding protein 2A
Zm00001d020019	12	li2013,liu2017,wang2018	Protein PHR1-LIKE 3
Zm00001d050669	7	li2013,liu2017,wang2018	bZIP protein
Zm00001d015521	11	li2013,liu2017,wang2018	Two-component response regulator ARR12
Zm00001d024220	8	li2013,liu2017	Putative HLH DNA-binding domain superfamily protein
Zm00001d024230	13	liu2017,wang2018	Nuclear transcription factor Y subunit C-2
Zm00001d044355	12	li2013,wang2018	B3 domain-containing protein
Zm00001d024268	14	liu2017,wang2018	NAC domain-containing protein 21/22
Zm00001d024041	13	liu2017,wang2018	bZIP transcription factor 16
Zm00001d045887	6	liu2017,wang2018	E3 ubiquitin-protein ligase makorin
Zm00001d044261	14	li2013,wang2018	Protein indeterminate-domain 9
Zm00001d011223	5	liu2017,wang2018	Protein indeterminate-domain 9
Zm00001d026448	8	li2013,wang2018	Floral homeotic protein APETALA 2
Zm00001d005029	8	liu2017,wang2018	Protein SCARECROW
Zm00001d020043	5	liu2017,wang2018	Ethylene-responsive transcription factor ERF117
Zm00001d044260	9	li2013,wang2018	C3H-type transcription factor
Zm00001d011252	5	liu2017,wang2018	Putative MYB-domain histone H1 family protein
Zm00001d040302	12	li2013,liu2017	Zinc finger CCCH type domain-containing protein ZFN-like 1
Zm00001d050242	9	liu2017,wang2018	Protein Dr1 homolog
Zm00001d041277	14	li2013,liu2017	Putative axial regulator YABBY 2
Zm00001d044242	17	li2013,wang2018	Transcription factor bHLH87
Zm00001d009599	5	liu2017,wang2018	Putative homeodomain-like transcription factor superfamily protein; SANT/MYB protein
Zm00001d050247	7	liu2017,wang2018	Putative WRKY DNA-binding domain superfamily protein; WRKY69-superfamily protein
Zm00001d043076	7	li2013,wang2018	WUSCHEL-related homeobox 13
Zm00001d041142	13	li2013,liu2017	bZIP transcription factor 16
Zm00001d020457	6	li2013,wang2018	Transcription factor MYB30
Zm00001d024679	13	li2013,wang2018	Transcription factor ILR3
Zm00001d049525	10	li2013,wang2018	LOL1; LOL1 isoform 1; LOL1 isoform 2
Zm00001d046774	9	li2013,wang2018	Zinc finger protein WIP2
Zm00001d050781	10	li2013,wang2018	Auxin response factor 1
Zm00001d046740	9	li2013,wang2018	Zinc finger CCCH domain-containing protein 52
Zm00001d015153	12	li2013,wang2018	BZIP transcription factor
Zm00001d046718	5	li2013,wang2018	bZIP transcription factor 60
Zm00001d036214	23	li2013,wang2018	B-box zinc finger protein 22
Zm00001d036148	14	li2013,wang2018	whirly1
Zm00001d010227	7	li2013,wang2018	Putative NAC domain transcription factor superfamily protein isoform 1; Putative NAC domain transcription factor superfamily protein
Zm00001d035439	11	liu2017	knotted related homeobox5
Zm00001d046323	7	liu2017	NA
Zm00001d044330	11	wang2018	Telomere repeat-binding factor 1
Zm00001d018465	18	wang2018	NA
Zm00001d046441	12	liu2017	FAR1-domain family sequence
Zm00001d027546	10	wang2018	Transcription factor bHLH93
Zm00001d035514	11	liu2017	Transcription factor IIIA
Zm00001d035470	12	liu2017	Putative MYB DNA-binding domain superfamily protein
Zm00001d035922	11	liu2017	Pentatricopeptide repeat-containing protein chloroplastic
Zm00001d017784	8	wang2018	Zinc-finger homeodomain protein 5
Zm00001d034091	19	wang2018	Transcription factor bHLH113
Zm00001d012605	9	wang2018	MRP interacting1
Zm00001d017788	10	wang2018	Dof zinc finger protein DOF2.1
Zm00001d035670	11	liu2017	GRAS transcription factor (Fragment) [Source:UniProtKB/TrEMBL;Acc:K7UES9]

Zm00001d048309	8	wang2018	DNA binding protein; Putative HLH DNA-binding domain superfamily protein
Zm00001d010842	5	liu2017	Pentatricopeptide repeat-containing protein
Zm00001d010785	6	liu2017	Putative GATA transcription factor family protein
Zm00001d027957	8	wang2018	MADS-transcription factor 68
Zm00001d026147	10	wang2018	plant color component at R1
Zm00001d012544	5	wang2018	myb domain protein 81
Zm00001d028007	7	wang2018	Calmodulin-binding transcription activator 2
Zm00001d033965	13	wang2018	Protein WRKY1
Zm00001d027709	6	wang2018	Transcription factor Dp-1
Zm00001d027530	8	wang2018	NAC-transcription factor 53
Zm00001d010758	5	liu2017	homeobox1
Zm00001d052192	12	li2013	Transcription factor bHLH99
Zm00001d048312	6	wang2018	Homeobox-leucine zipper protein ATHB-23
Zm00001d003822	11	wang2018	DNA-binding protein S1FA
Zm00001d026351	7	wang2018	Homeodomain leucine zipper family IV protein; Putative homeobox/lipid-binding do
Zm00001d033945	10	wang2018	Diphosphomevalonate decarboxylase MVD2
Zm00001d048404	12	wang2018	Probable lysine-specific demethylase ELF6
Zm00001d003616	5	liu2017	Zinc finger CCCH domain-containing protein 28
Zm00001d006677	18	li2013	Protein BRASSINAZOLE-RESISTANT 1
Zm00001d047519	7	wang2018	basic leucine-zipper 52
Zm00001d030897	6	wang2018	NA
Zm00001d044168	12	li2013	Heat stress transcription factor C-1
Zm00001d053162	7	wang2018	Transcription factor VIP1
Zm00001d003162	24	li2013	Zinc finger protein CONSTANS-LIKE 5
Zm00001d020490	6	li2013	Transcription factor bHLH69
Zm00001d047644	6	wang2018	Putative bZIP transcription factor superfamily protein
Zm00001d006701	24	li2013	GRAS transcription factor [Source:UniProtKB/TrEMBL;Acc:C0P6K9]
Zm00001d029934	5	wang2018	Homeobox-leucine zipper protein HAT4
Zm00001d021927	6	li2013	BES1/BZR1 protein
Zm00001d015412	6	wang2018	sequence-specific DNA binding transcription factors
Zm00001d030727	7	wang2018	Dof zinc finger protein DOF2.2
Zm00001d021701	5	wang2018	Transcription factor ILR3
Zm00001d053988	7	li2013	Ocs element-binding factor 1; Putative bZIP transcription factor superfamily protein
Zm00001d053178	7	wang2018	Trihelix transcription factor [Source:UniProtKB/TrEMBL;Acc:C0PKA0]
Zm00001d006578	17	li2013	Transcription factor bHLH130
Zm00001d029963	6	wang2018	myb-like transcription factor family protein
Zm00001d006619	8	li2013	Zinc finger CCCH domain-containing protein 56
Zm00001d020408	8	wang2018	Typical P-type R2R3 Myb protein
Zm00001d030908	6	wang2018	B3 domain-containing protein
Zm00001d043500	5	wang2018	Transcription factor VOZ1
Zm00001d006565	15	li2013	male sterile32
Zm00001d047563	8	wang2018	ethylene insensitive-like1
Zm00001d030907	6	wang2018	B3 domain-containing protein
Zm00001d052180	7	li2013	tunicate1
Zm00001d052798	8	li2013	Homeodomain-like superfamily protein
Zm00001d028504	6	li2013	Transcription factor AIG1
Zm00001d020881	6	li2013	Probable WRKY transcription factor 34
Zm00001d051269	7	wang2018	myb domain protein 17
Zm00001d006835	9	li2013	Nuclear transcription factor Y subunit A-8
Zm00001d006682	6	li2013	Protein indeterminate-domain 11
Zm00001d016255	13	li2013	Heat stress transcription factor C-1
Zm00001d013547	9	li2013	BEL1-like homeodomain protein 3
Zm00001d007465	8	li2013	Growth-regulating factor 2
Zm00001d019230	6	wang2018	sister of indeterminate spikelet1

Zm00001d013465	6	li2013	Dwarf plant9
Zm00001d031109	5	wang2018	Putative axial regulator YABBY 2
Zm00001d030891	5	wang2018	Homeodomain-like superfamily protein
Zm00001d052847	8	wang2018	Putative WRKY DNA-binding domain superfamily protein
Zm00001d016154	7	li2013	Basic leucine zipper 34
Zm00001d036536	20	li2013	Ethylene-responsive transcription factor 7
Zm00001d036632	18	li2013	Protein REVEILLE 6
Zm00001d014377	7	wang2018	Auxin response factor 18
Zm00001d006451	7	li2013	Squamosa promoter-binding-like protein 5
Zm00001d036692	19	li2013	SBP-domain protein3
Zm00001d051140	7	wang2018	NAC domain containing protein 74
Zm00001d042463	14	li2013	Two-component response regulator ARR11
Zm00001d014995	9	wang2018	Transcription factor bHLH104
Zm00001d051439	8	li2013	Dof zinc finger protein DOF2.5
Zm00001d014723	8	wang2018	NA
Zm00001d030028	5	wang2018	myc transcription factor7
Zm00001d035965	6	li2013	Growth-regulating factor 6
Zm00001d049347	7	li2013	Zinc finger protein CONSTANS-LIKE 5
Zm00001d046564	9	li2013	Putative bZIP transcription factor superfamily protein [Source:UniProtKB/TrEMBL;

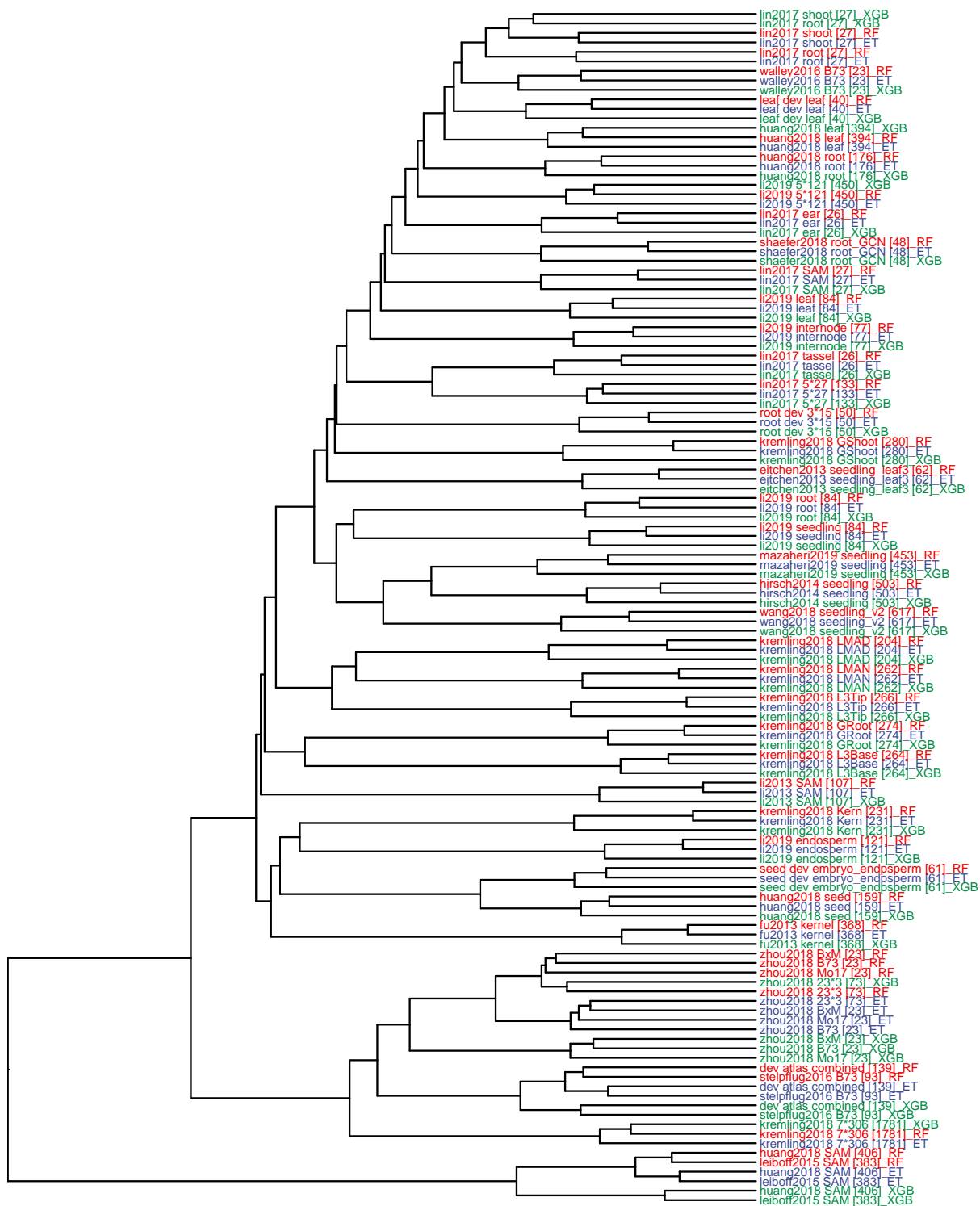


Figure S1. Hierarchical clustering of 126 GRNs (42 expression dataset * 3 methods). Each of the three regression-based method (random forest, extra trees, gradient boosted) was used to build a network for each RNA-Seq dataset. Pairwise distance between networks was determined by taking the top 100,000 TF-target predictions from each network and calculating the proportion of shared (common) predictions (using ‘dist()’

function in R with additional argument 'method=binary'). Hierarchical clustering was then performed based on the cross-network pairwise distance matrix using "ward.D" option.

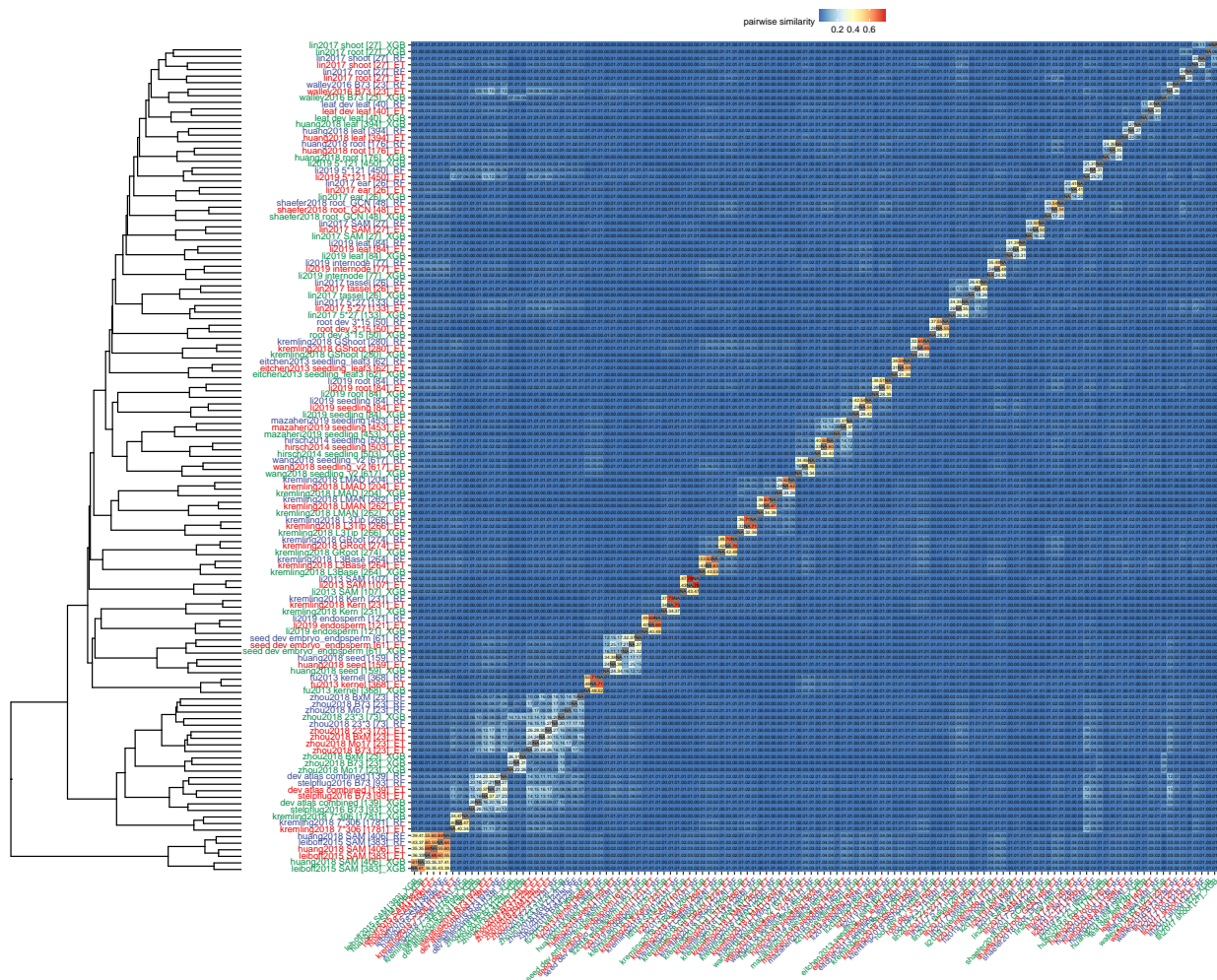


Figure S1b.

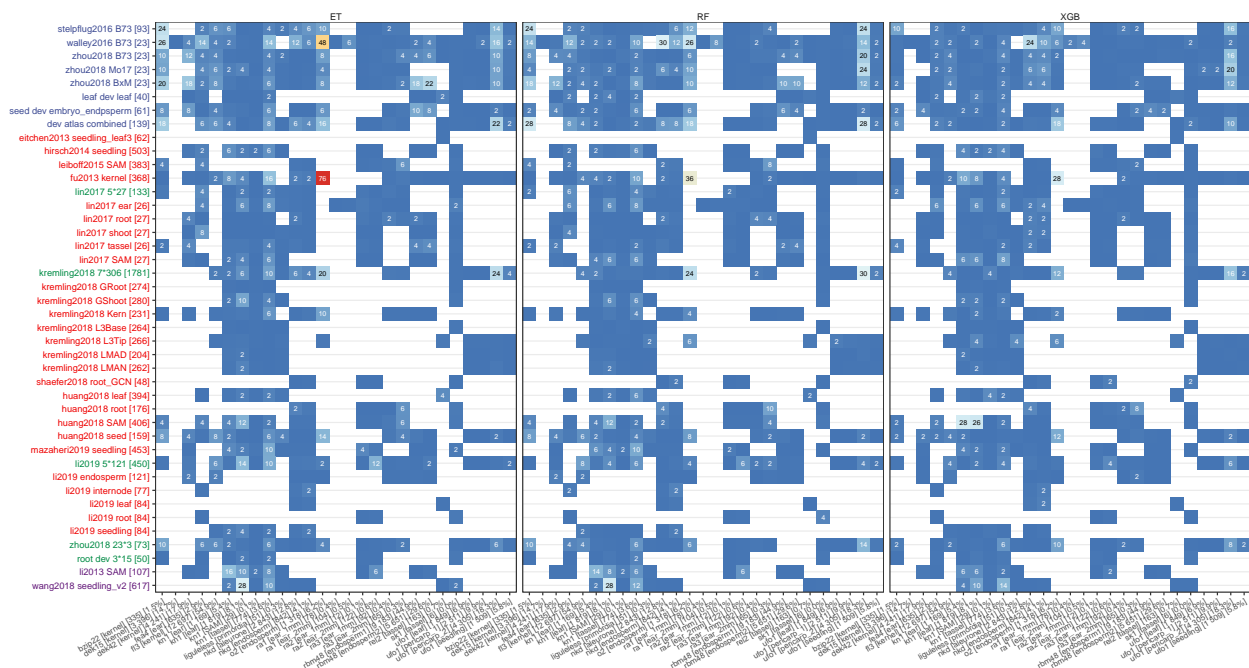


Figure S3. Comparison of GRNs built with different methods using supports from TF knockout mutant RNA-Seq data.

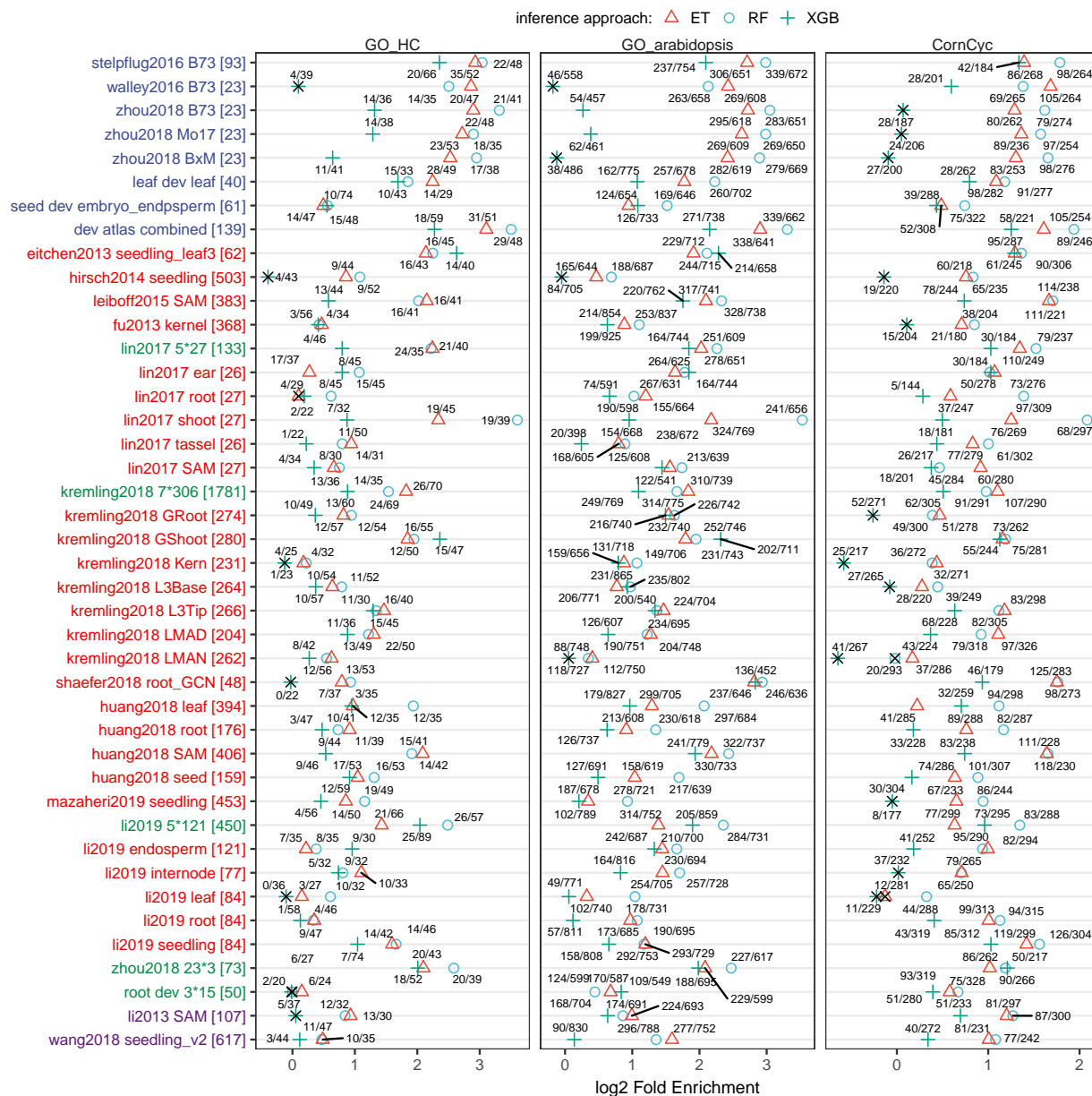
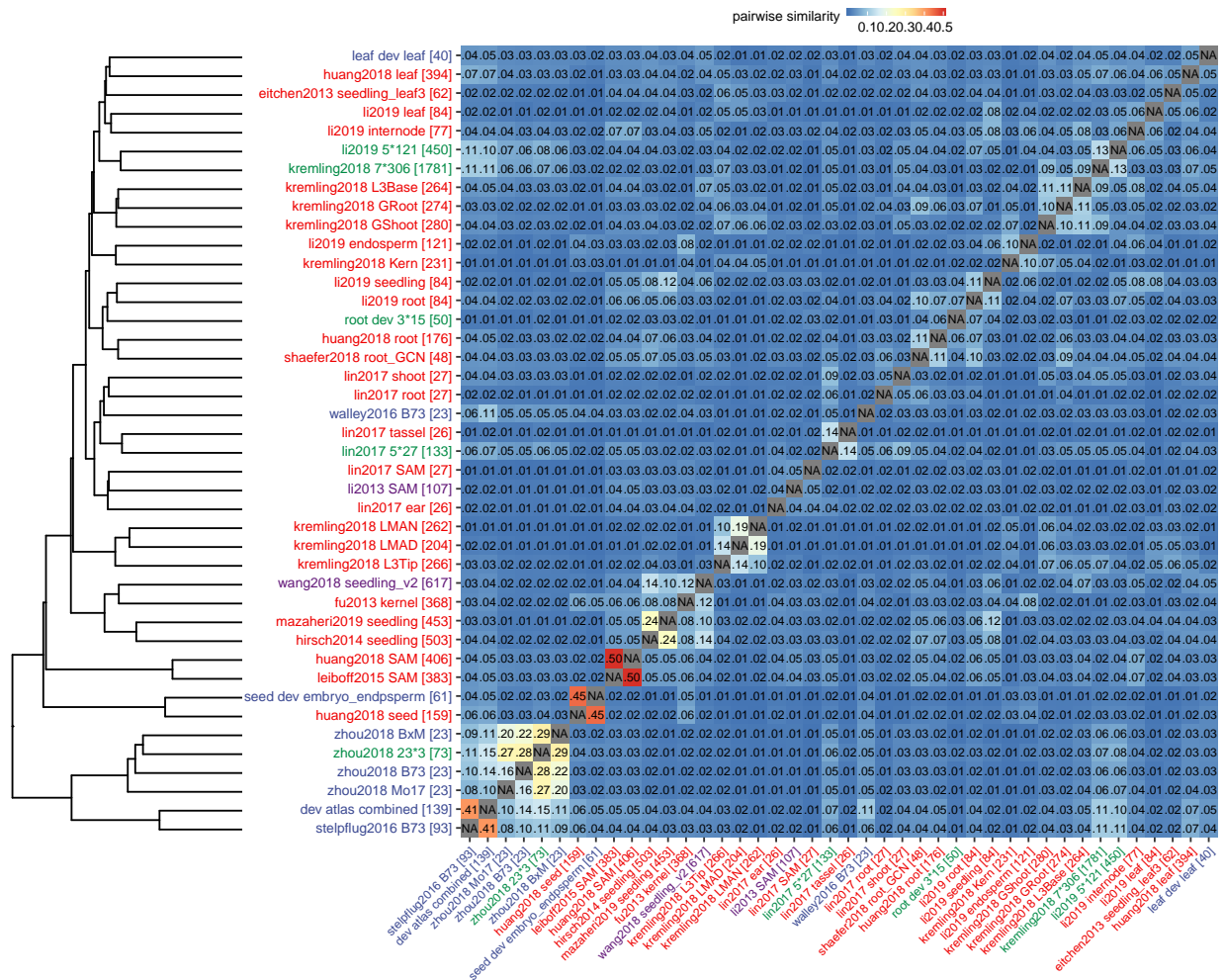


Figure S4. Comparison of GRNs built with different methods using supports from TF knockout mutant RNA-Seq data.



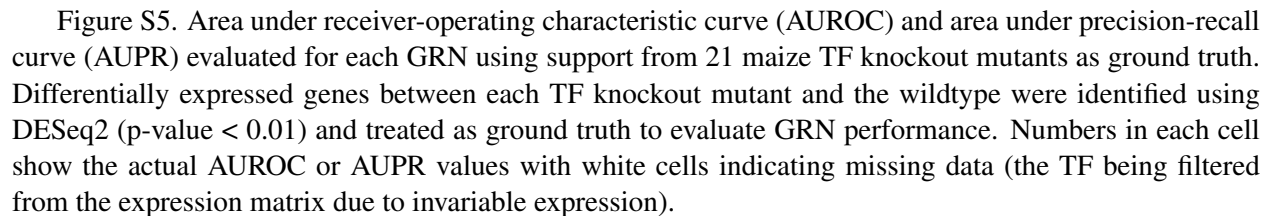


Figure S5. Area under receiver-operating characteristic curve (AUROC) and area under precision-recall curve (AUPR) evaluated for each GRN using support from 21 maize TF knockout mutants as ground truth. Differentially expressed genes between each TF knockout mutant and the wildtype were identified using DESeq2 (p-value < 0.01) and treated as ground truth to evaluate GRN performance. Numbers in each cell show the actual AUROC or AUPR values with white cells indicating missing data (the TF being filtered from the expression matrix due to invariable expression).

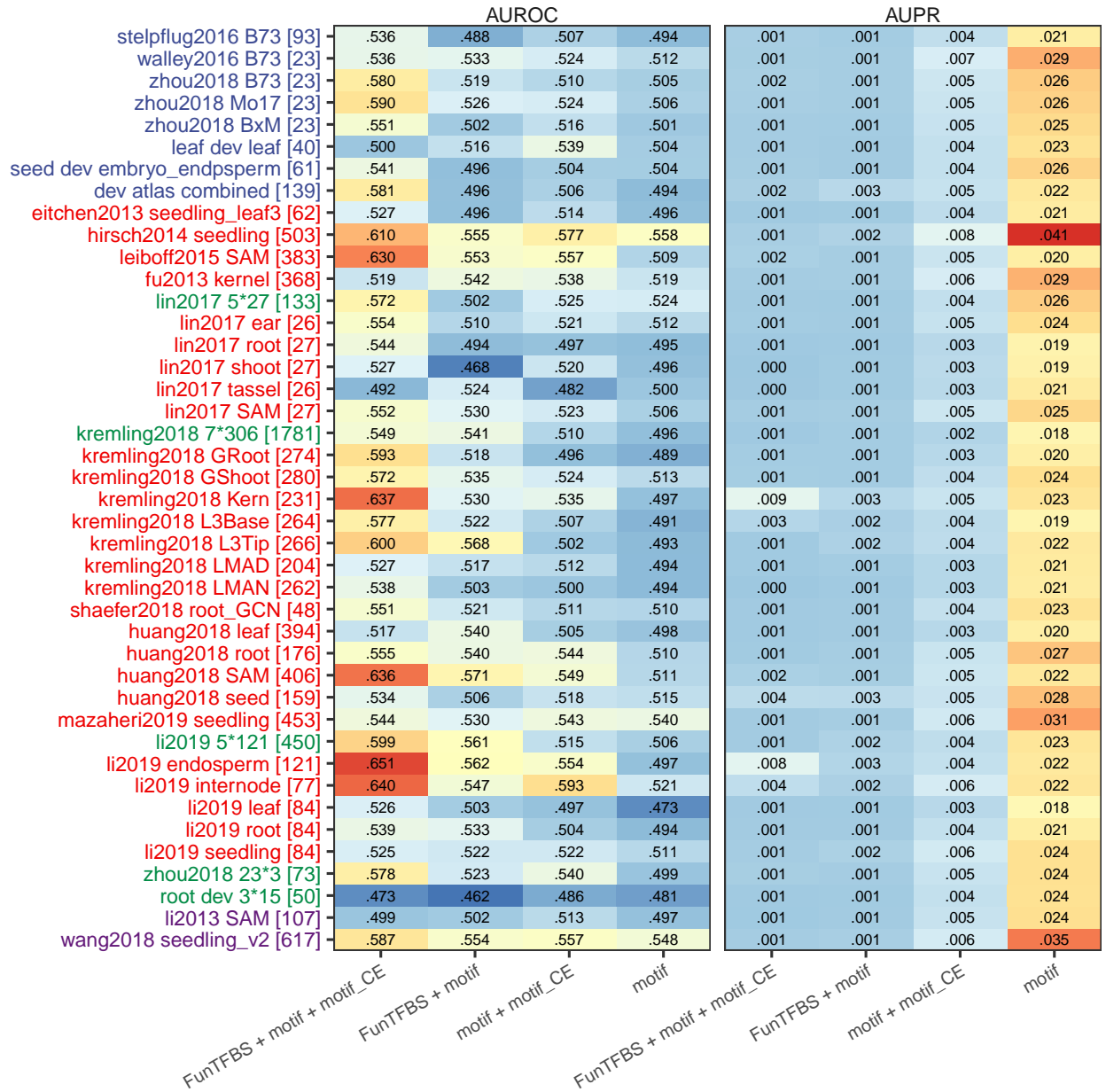


Figure S6. Evaluation of constructed GRNs using four sets of predicted TF-target interactions based on TF-binding site motif, conserved element of TFBS motif or FunTFBS. There are 202,670 “motif”-based predictions, 37,699 predictions based on motif and cross-species conservation, 7,459 predictions based on motif and FunTFBS as well as 4,115 predictions based on all three evidences.

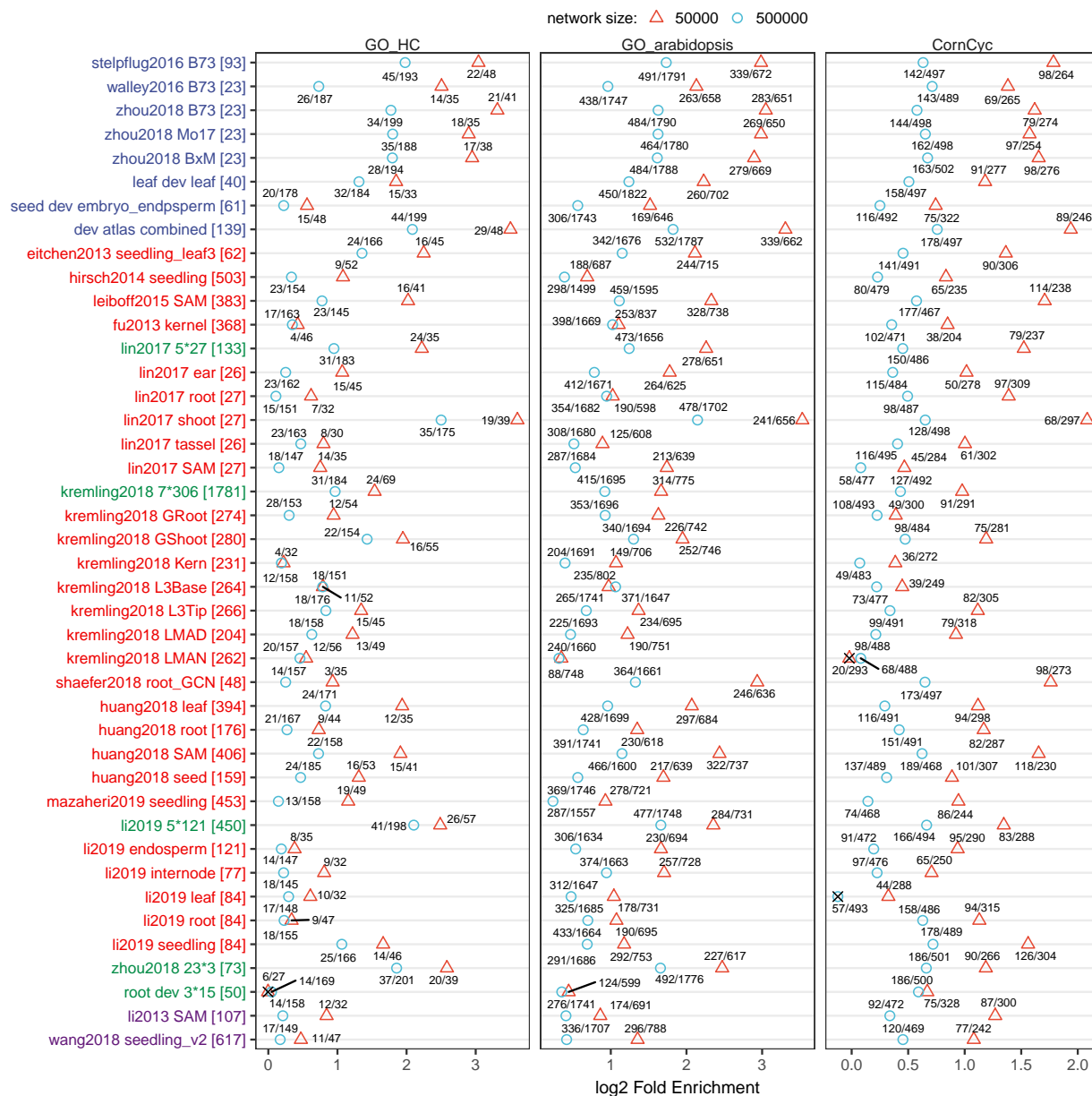
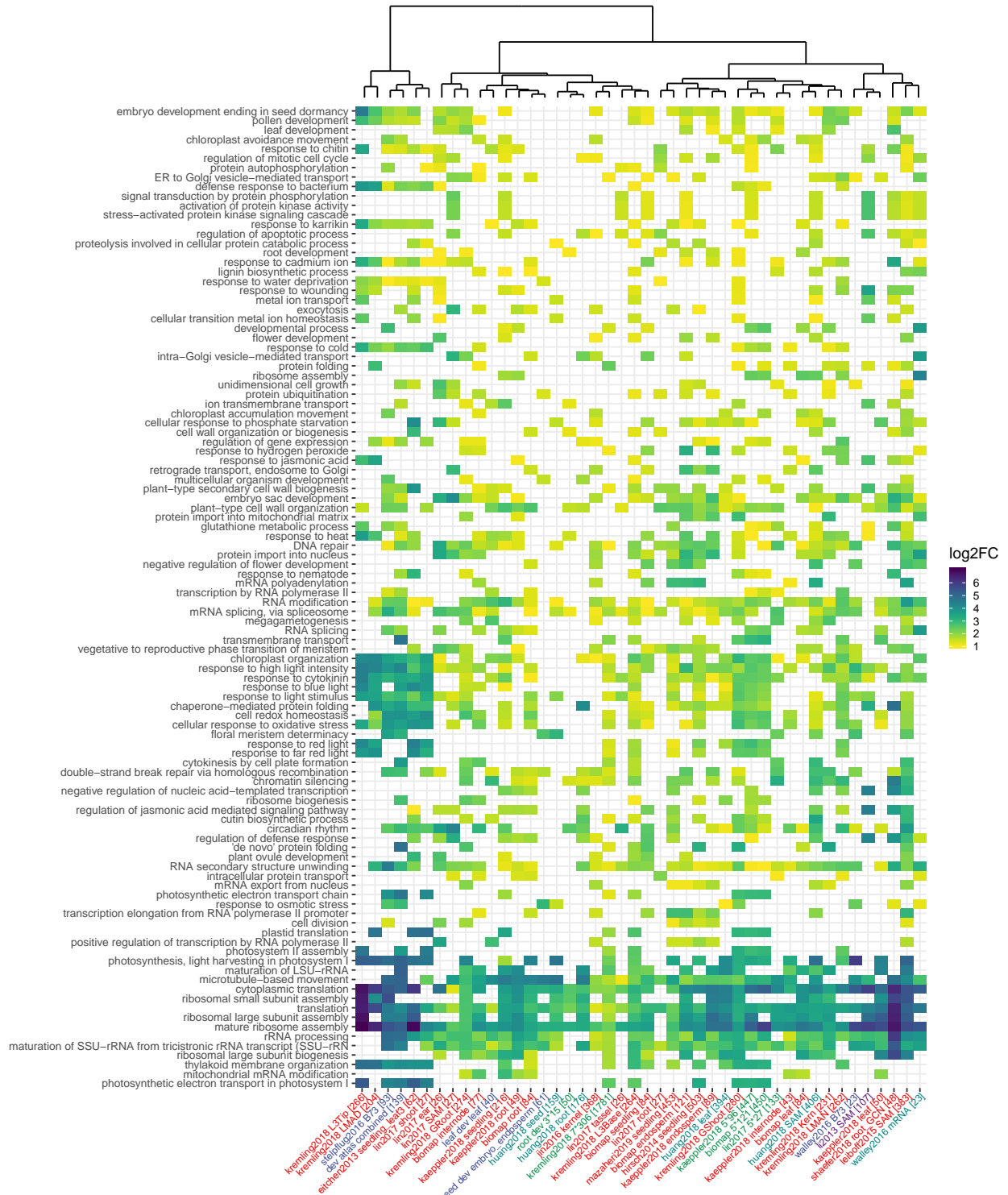


Figure 3. Enrichment of co-annotated GO/CornCyc terms in co-regulated network targets. For each network either the top 50,000 edges (red triangle) or 500,000 edges (blue circle) was taken to assess enrichment of GO/CornCyc annotation. Log2 fold enrichment is calculated as the observed number of shared GO/CornCyc terms (by targets regulated by a common TF) divided by the expected number of shared annotation terms (determined by permutation). The names for each of the networks are color coded to indicate B73 developmental surveys (blue), genotype surveys (red), meta-networks (green) or previously generated GRNs (teal).



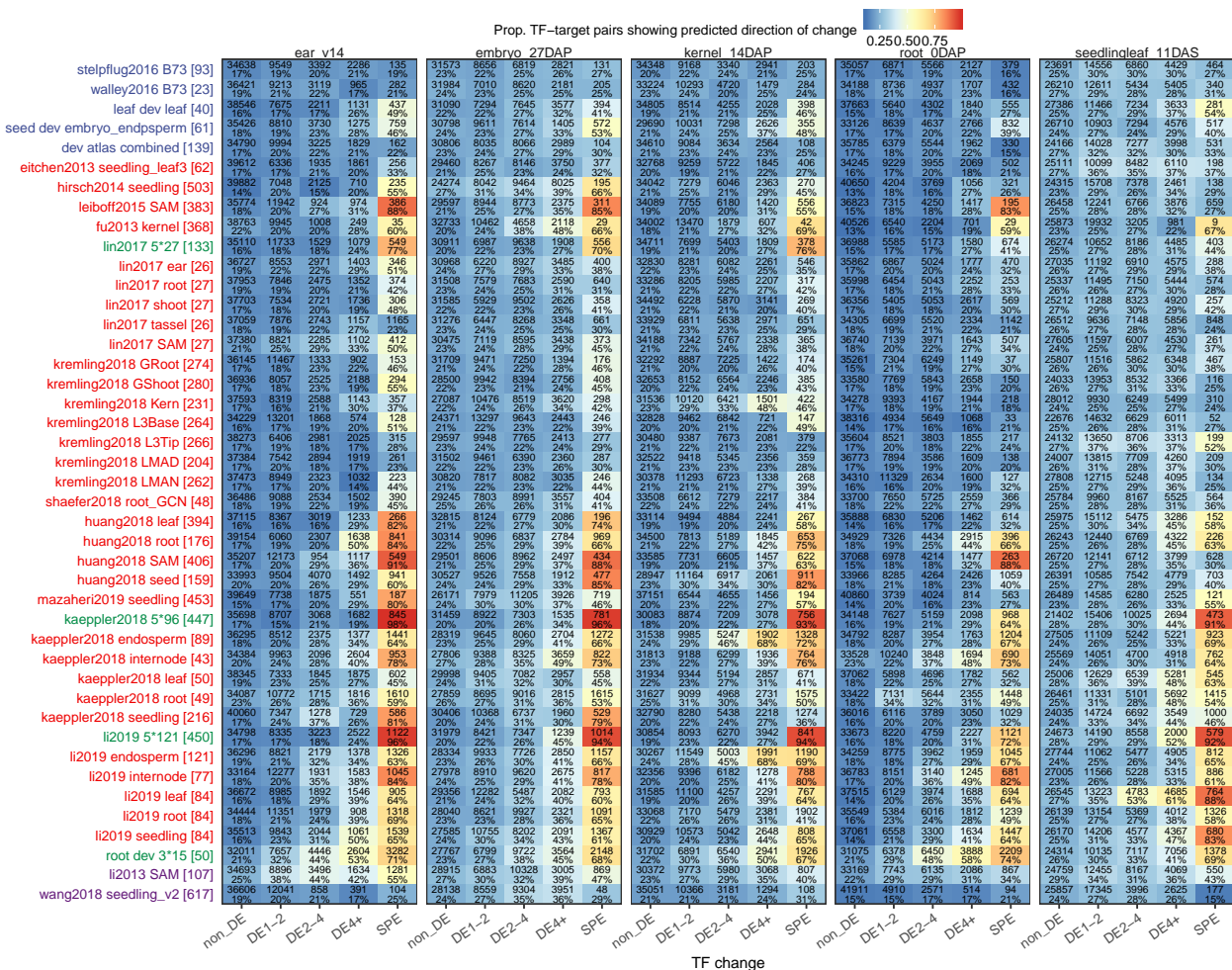


Figure S7. Proportion of differentially expressed targets regulated by TFs showing different DE levels. Each TF–target pair is classified according to the DE level of the TF (“non_DE”, “DE1-2”, “DE2-4”, “DE4+” or “SPE”) in each network. The proportion of TF–target pairs with the target also showing DE was then determined for each category. Numbers in each cell show the total number of TF–target pairs falling in that category as well as the actual proportion showing predicted direction of changes.

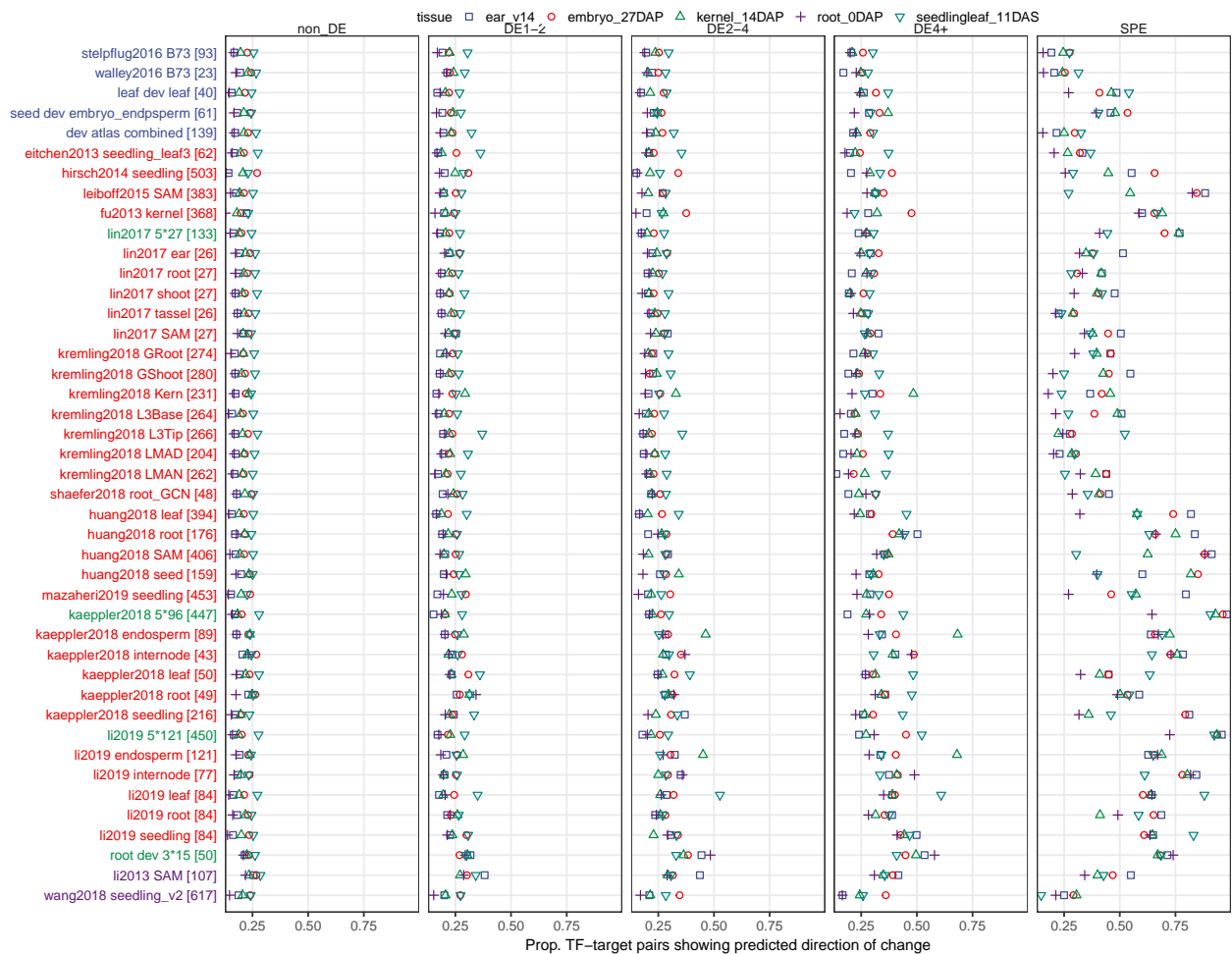


Figure S7b. Another way of showing Figure S7.

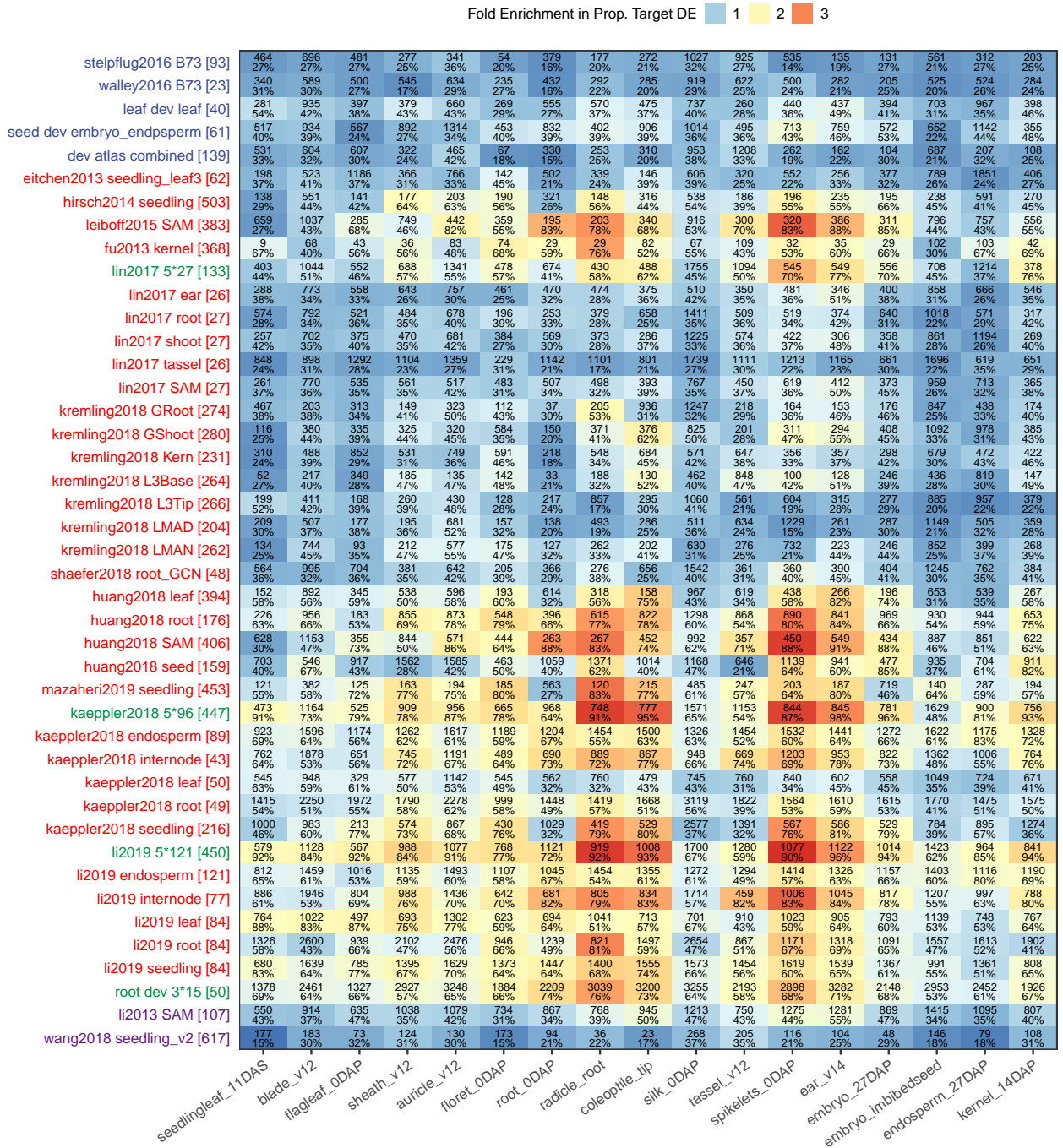


Figure 5. Enrichment in differentially expressed targets regulated by TFs that show SPE patterns. Color in each cell represents the fold enrichment of (SPE TF regulated) target DE proportions relative to the tissue-wise average proportion of DEGs. Number in each cell shows total number of TF-target pairs regulated by TF showing SPE in that tissue as well as the actual proportion showing predicted direction of changes.

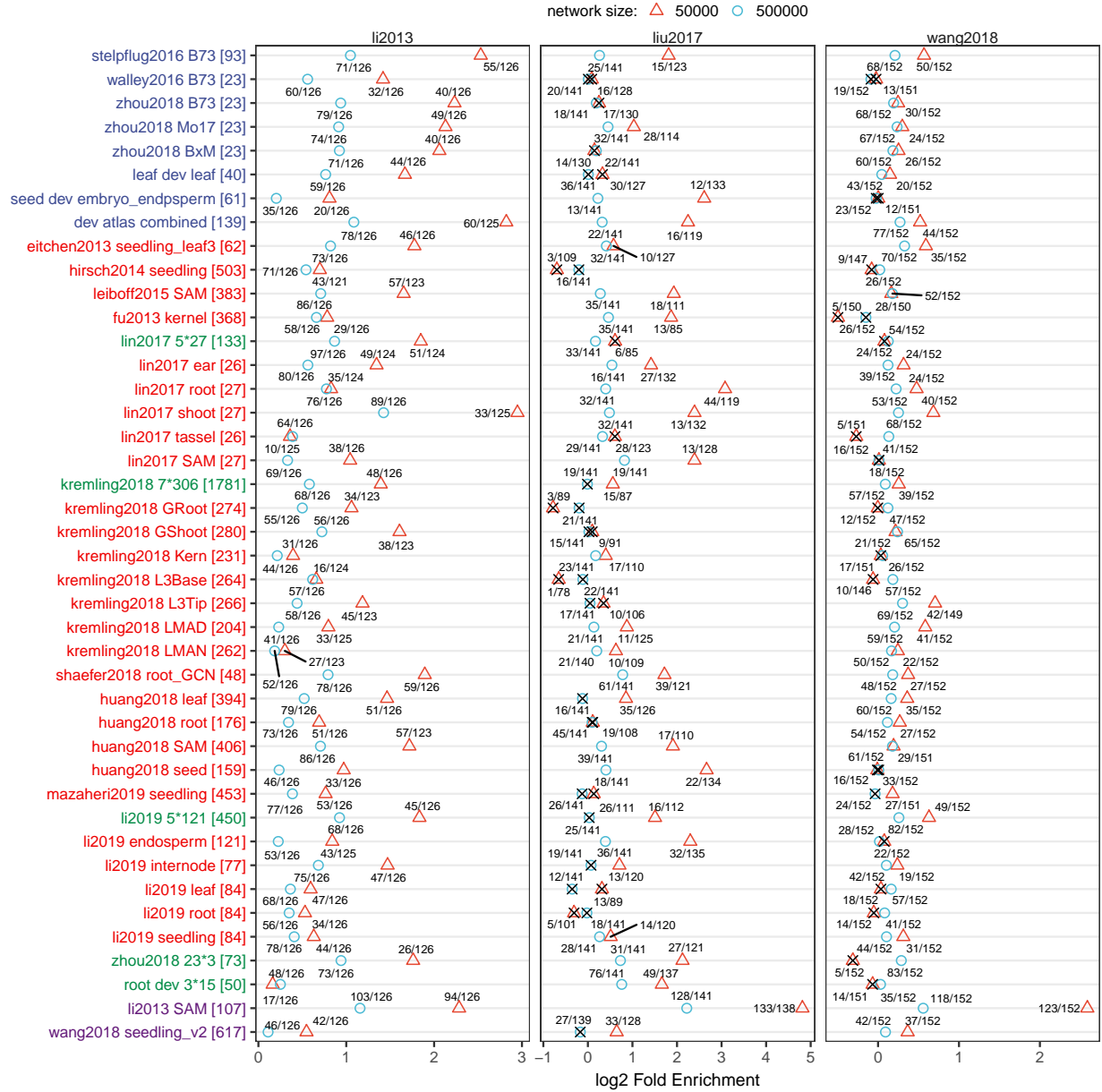


Figure S8. Enrichment of co-regulated targets between known trans-eQTL hotspots (identified in previous studies) and GRN-predicted TF-target associations. For each network either the top 50,000 edges (red triangle) or 500,000 edges (blue circle) was taken to assess enrichment of co-regulation. Log2 fold enrichment is determined by the same permutation approach described in Figure B.

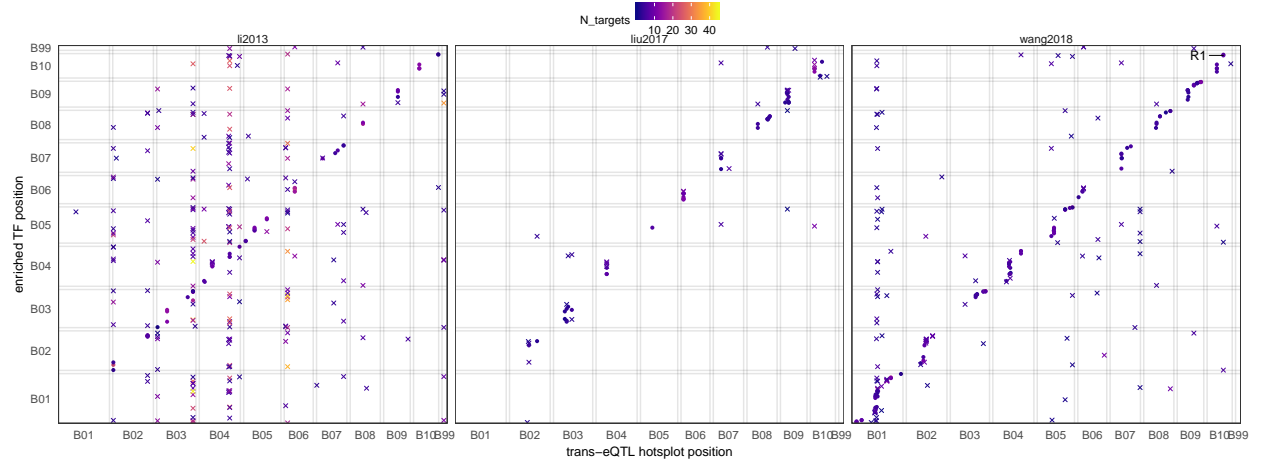


Figure 6. Identification of acting transcription factors underlying trans-eQTL hotspots in previous eQTL studies. For each trans-eQTL hotspot identified in three previous eQTL studies, the physical genomic locations were extracted and converted to AGPv4 coordinates, and the regulated targets (i.e., eTraits) for each trans-eQTL hotspot were also converted to AGPv4 gene IDs. Each dot stands for a significant co-regulation between a trans-eQTL hotspot (x-axis) and a TF in GRN (y-axis) with the level of significance determined in Figure F. Color of dot represents the number of common targets between the predicted TF regulator and the trans-eQTL hotspot.