

Supplemental Figure 1: SL signalling components function in the nucleus

**A-B)**: Maximum intensity confocal projections of fields of *N. benthamiana* epidermal cells expressing D14-CFP (A) and SMXL7-YFP (B) from the 35S promoter.

**C-E)**: Sub-cellular localization of SMXL7-YFP (C) and SMXL7DNLS-YFP (D-E) expressed in *N. benthamiana* epidermal cells from the 35S promoter. C and D are single confocal slices, E is a maximum intensity projection of multiple slices through the same cell shown in D.

**F-G)** Localization of SMXL7<sup> $\Delta$ NLS</sup>-YFP in an Arabidopsis root meristem at 0 and 20 minutes after treatment with 5 $\mu$ M *rac*-GR24. Image F is also shown in Figure 2. Plants are from a homozygous transgenic line in which the fusion protein is expressed from the 35S promoter.

**H-J)**: Co-localization of SMXL7-YFP (H) and D14-CERULEAN (I) in an Arabidopsis root; merged image in (J). Plants are homozygous double transgenic lines in which the fusion proteins are expressed from the 35S promoter.

**K)** Rosette branching levels in Col-0, *d14-1*, *d14-1* D14:D14-CERULEAN (9 independent homozygous lines), *d14-1* D14:D14<sup>NLS</sup>-VENUS (9 independent homozygous lines) and *d14-1* D14:D14<sup>Palm/myr</sup>-CERULEAN (10 independent homozygous lines). Plants were grown in short days for 4 weeks, then grown in long days until the inflorescence stem was 10cm long, and then decapitated. Primary rosette branches were counted 10 days later (Greb et al, 2003). Extracts of this complete dataset are shown in Figure 2. n=13-20 error bars indicate s.e.m.



# Supplemental Figure 2: SMXL7 physically interacts with D14 in a SL-dependent manner

**A)** Controls for Figure 3, showing lack of FRET in *N. benthamiana* epidermal cells singly-transfected with D14-CFP and MAX2-CFP as visualized by FLIM (top and middle row), and showing lack of FRET between MAX2-CFP and SMXL7<sup>d53</sup>-YFP (bottom row). See Figure 3 for more detail. Scale bars indicate  $5\mu$ M.

**B)** Detection of FRET between SMXL6-CFP and SMXL7-YFP using spectral analysis in doubly-transfected *N. benthamiana* epidermal cells, driven by the *35S* promoter. SMXL6 and SMXL7 co-localize in the nucleus. The SMXL6-CFP in four randomly selected regions of interest (ROI) was excited using a 405 nm laser line, and the resulting emission spectrum recorded. In addition to the expected CFP emission spectrum (dotted line) measured from a cell expressing only SMXL6-CFP, each region of interest showed an emission peak at 520-530 nm, consistent with emission of YFP-type spectra (coloured lines), implying FRET between SMXL6 and SMXL7.

**C)** Co-localization of SMXL6-mCherry and SMXL7-YFP in an Arabidopsis root meristem, and co-degradation in response to 10 minutes treatment with  $5\mu$ M rac-GR24 (bottom row). Plants are doubly homozygous for transgenes driving expression of the fusion proteins from the *35S* promoter. Scale bar indicates  $100\mu$ M.



## Supplemental Figure 3: Effect of SMXL7 dose on development.

**A-F):** Adult shoot morphology in Col-0 (A,B), *d14-1* (C,D) and *max1-1* (E,F) untransformed (A,C,E) or homozygous for *SMXL7*-*VENUS* (B,D,F).

**G)**: Branching angle in Col-0, smxl6-4 smxl7-3 smxl8-1 max2-1 and max2-1 untransformed ('-') or homozygous for  $SMXL7_{pro}$ : SMXL7-VENUS ('+'). n=18-39, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**H)**: Height of the primary inflorescence stem in Col-0, *smxl6-4 smxl7-3 smxl8-1 max2-1* and *max2-1* untransformed ('-') or homozygous for SMXL7-VENUS ('+'), measured at proliferative arrest. n=20-24, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).



# Supplemental Figure 4: Effect of SMXL7 stabilization on development.

**A)**: Branching angle in untransformed Col-0, Col-0 transformed with  $SMXL7_{pro}:SMXL7-VENUS$ ,  $SMXL7_{pro}:SMXL7^{d53}-VENUS$ ,  $SMXL7_{pro}:SMXL7^{d53}-VENUS$ ,  $SMXL7^{d53}-VENUS$ ,  $SMXL7^{d53}-V$ 

**B)**: Height of the primary inflorescence stem in untransformed Col-0, Col-0 transformed with  $SMXL7_{pro}:SMXL7-VENUS$ ,  $SMXL7_{pro}:SMXL7^{d^{53}}-VENUS$ ,  $SMXL7^{d^{53}}-VENUS$ ,  $SMXL7^{d^{P-loop}}-VENUS$ ,  $35S_{pro}:SMXL7^{d^{53}}-YFP$ ,  $35S_{pro}:SMXL7^{d^{P-loop}}-YFP$  (hemizygous), and in d14-1, measured at proliferative arrest. n=10, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).



# Supplemental Figure 5: Effects of SMXL7 are partially EAR-independent

**A)**: Branching angle in Col-0, *max2-1*, untransformed *smxl6-4 smxl7-3 smxl8-1 max2-1* (s678m2) or s678m2 homozygous for *SMXL7<sub>pro</sub>:SMXL7-VENUS*, *SMXL7<sub>pro</sub>:SMXL7<sup>alaEAR</sup>-VENUS* and *SMXL7<sub>pro</sub>:SMXL7<sup>4EAR</sup>-VENUS*. n=12-16, bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**B)**: Diameter ( $\mu$ m) of the basal internode of the primary inflorescence stem in Col-0, *max2-1*, untransformed *smxl6-4 smxl7-3 smxl8-1 max2-1* (*s678m2*) or *s678m2* homozygous for *SMXL7:SMXL7-VENUS*, *SMXL7*<sub>pro</sub>:*SMXL7*<sup>alaEAR</sup>-*VENUS* and *SMXL7*<sub>pro</sub>:*SMXL7*<sup>dEAR</sup>-*VENUS*, measured at proliferative arrest. n=12-16, bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

## Supplemental Data. Liang et al. Plant Cell (2016) 10.1105/tpc.16.00286



## Supplemental Figure 6: Effects of SMXL7 are partially EAR-independent

**A-E):** Rosette leaf morphology in Col-0 (A), *max2-1* (B), untransformed *smxl6-4 smxl7-3 smxl8-1 max2-1* (*s678m2*) (C) or *s678m2* homozygous for *35S*<sub>pro</sub>:*SMXL7-YFP* (D) or *35S*<sub>pro</sub>:*SMXL7<sup>alaEAR</sup>-YFP* (E).

**F)**: Adult shoot morphology in *max2-1*, untransformed *smxl6-4 smxl7-3 smxl8-1 max2-1* (s678m2) or s678m2 homozygous for 35S<sub>pro</sub>:SMXL7-YFP or 35S<sub>pro</sub>:SMXL7<sup>alaEAR</sup>-YFP.

**G)**: Leaf blade length, blade width and petiole length in the 7<sup>th</sup> leaf of Col-0, *max2-1*, untransformed *smxl6-4* smxl7-3 smxl8-1 max2-1 (s678m2) or s678m2 homozygous for  $35S_{pro}$ : SMXL7-YFP or  $35S_{pro}$ : SMXL7<sup>alaEAR</sup>-YFP. n=10-11, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**H)**: Number of cauline, rosette and total primary branches of Col-0, *max2-1*, untransformed *smxl6-4 smxl7-3* smxl8-1 max2-1 (s678m2) or s678m2 homozygous for with  $35S_{pro}$ :SMXL7-YFP or  $35S_{pro}$ :SMXL7<sup>alaEAR</sup>-YFP. n=9-11, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**I)**: Branching angle in Col-0, *max2-1*, untransformed *smxl6-4 smxl7-3 smxl8-1 max2-1* (*s678m2*) or *s678m2* homozygous for  $35S_{pro}:SMXL7-YFP$  or  $35S_{pro}:SMXL7^{alaEAR}-YFP$ . n=17-20, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**J**): Height of the primary inflorescence stem in Col-0, *max2-1*, untransformed *smxl6-4 smxl7-3 smxl8-1* max2-1 (s678m2) or s678m2 homozygous for  $35S_{pro}$ : SMXL7-YFP or  $35S_{pro}$ : SMXL7<sup>alaEAR</sup>-YFP, measured at proliferative arrest. n=9-11, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).



### Supplemental Figure 7: Loss of the EAR motif counteracts SMXL7 stabilization

**A):** Branching angle in Col-0 and *smxl678*, either untransformed or homozygous for  $SMXL7_{pro}$ :  $SMXL7^{d53}$ -VENUS or  $SMXL7_{pro}$ :  $SMXL7^{d53,alaEAR}$ -VENUS. n=16-23, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**B):** Height of the primary inflorescence stem in Col-0 and *smxl678*, either untransformed or homozygous for  $SMXL7_{pro}:SMXL7^{d53}-VENUS$  or  $SMXL7_{pro}:SMXL7^{d53,alaEAR}-VENUS$ , measured at proliferative arrest. n=10, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).

**C):** Diameter of the basal internode of the primary inflorescence stem in Col-0 and *smxl678*, either untransformed or homozygous for  $SMXL7_{pro}$ :  $SMXL7^{d53}$ -VENUS or  $SMXL7_{pro}$ :  $SMXL7^{d53,alaEAR}$ -VENUS, measured at proliferative arrest. n=11-15, error bars indicate s.e.m, bars with the same letter are not significantly different from one another, (ANOVA + Tukey HSD, p<0.05).



# Supplemental Figure 8: Loss of the EAR motif counteracts SMXL7 stabilization

**A):** Rosette leaf morphology in untransformed Col-0, or Col-0 homozygous for  $35S_{pro}$ :SMXL7<sup>AP-loop</sup>-YFP or  $35S_{pro}$ :SMXL7<sup>AP-loop</sup>-YFP.

**B)**: Adult shoot morphology in Col-0, or Col-0 homozygous for  $35S_{pro}$ : SMXL7<sup>4P-loop</sup>-YFP or  $35S_{pro}$ : SMXL7<sup>4P-loop</sup>-YFP.

Variant	Position (aa)	Modification	Primers
SMXL7 <sup>ΔNLS</sup>	85 to 117	deleted	F: CAACGACGTCATCCAGAG R: GTCTAGAGAAACGCCGAC
SMXL7 <sup>ΔNTP1</sup>	624 to 796	deleted	F: GAACCAGTCAAATACTCCG R: TCTCGAAGCTGACAACAATTG
SMXL7 <sup>ΔP-loop</sup>	718 to 725	deleted	F :TACATTGCTGGCGAAGTG R: TCTATCGTCAAGACTGTCTTG
SMXL7 <sup>d53</sup>	719 to 724	$RGKTVV\toT$	F: AGATTACATTGCTGGCGAAGTG R: GTGAATCTATCGTCAAGACTGTCTTG
SMXL7 <sup>∆EAR</sup>	854 to 860	deleted	F: GATGAGATTGAAGCAAACG R: AAACGAACGCTGAGACTTAAG
SMXL7 <sup>alaEAR</sup>	854,856,858	LDLNLP → ADANAP	F: TGCTCCTGTGGATGAGATTGAAGCAAACG R: TTCGCATCTGCAAACGAACGCTGAGACTTAAG
$SMXL7^{\DeltaP ext{-loop},\DeltaEAR}$	718 to 725 854 to 860	deleted	the same as SMXL7 <sup><math>\Delta</math>P-loop</sup> and S7 <sup><math>\Delta</math>EAR</sup>
SMXL7 <sup>d53, alaEAR</sup>	719 to 724 854,856,858	$\begin{array}{l} RGKTVV \to T, \\ LDLNLP \to \\ ADANAP \end{array}$	the same as SMXL7 <sup>alaEAR</sup> and SMXL7 <sup>d53</sup>
SMXL7 <sup>ΔNTP2</sup>	871 to 930	deleted	<i>F</i> : ATCCTTAAGATTCTTGCTGC <i>R</i> : CGCTTCGTCTTCGTTTGC
D14 <sup>palm/myr</sup>	after 1	added GGCFSKK	F: AGCAAGAAGAGTCAACACAACATCTT AGAAGCTCTAAATGTCC R: GAAACAGCCATTAAGCCATTAAGCCT GCTTTTTTGTACAAACTTTGG

# Supplemental Table 1

Protein variants created in this study, indicating the position and type of modification, and the primers used for site-directed mutagenesis to create these variants.

Construct	Method	Binary vector	Species
35Spro:SMXL7-YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}$ :SMXL7 <sup><math>\Delta</math>NLS</sup> -YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}$ :SMXL7 <sup><math>\Delta</math>NTP1</sup> -YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}$ :SMXL7 <sup><math>\Delta P</math>-loop</sup> -YFP	Gateway	pEarleyGate101	Nb, At
35Spro:SMXL7 <sup>d53</sup> -YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}$ :SMXL7 <sup><math>\Delta</math>EAR</sup> -YFP	Gateway	pEarleyGate101	Nb, At
35S <sub>pro</sub> :SMXL7 <sup>alaEAR</sup> -YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}:SMXL7^{\Delta P-loop,\Delta EAR}$ -YFP	Gateway	pEarleyGate101	Nb, At
$35S_{pro}$ :SMXL7 <sup><math>\Delta</math>NTP2</sup> -YFP	Gateway	pEarleyGate101	Nb, At
35Spro:SMXL7-CFP	Gateway	pEarleyGate102	Nb
SMXL7 <sub>pro</sub> :SMXL7-VENUS	Gateway	pB7m34GW,0	At
$SMXL7_{pro}:SMXL7^{\Delta NLS} - VENUS$	Gateway	pB7m34GW,0	At
$SMXL_{pro}:SMXL7^{\Delta P-loop} - VENUS$	Gateway	pB7m34GW,0	At
SMXL7 <sub>pro</sub> :SMXL7 <sup>d53</sup> -VENUS	Gateway	pB7m34GW,0	At
$SMXL_{pro}:SMXL7^{\Delta EAR}$ - VENUS	Gateway	pB7m34GW,0	At
SMXL7 <sub>pro</sub> :SMXL7 <sup>alaEAR</sup> - VENUS	Gateway	pB7m34GW,0	At
$SMXL_{pro}:SMXL7^{d53,alaEAR}$ -VENUS	Gateway	pB7m34GW,0	At
SMXL7 <sub>pro</sub> :GUS	Gateway	pH7m24GW,3	At
35Spro:SMXL6-CFP	Gateway	pEarleyGate102	Nb
35S <sub>pro</sub> :SMXL6-mCherry	Gateway	pK7m34GW,0	At
35S <sub>pro</sub> :D14-CFP	Gateway	pEarleyGate102	Nb
35S <sub>pro</sub> :D14-YFP	Gateway	pEarleyGate101	Nb
35Spro:D14-CERULEAN	Gateway	pH7m34GW,0	Nb, At
35Spro:D14 <sup>NLS</sup> -VENUS	Gateway	pH7m34GW,0	Nb, At
35S <sub>pro</sub> :D14 <sup>palm/myr</sup> -GFP	Gateway	pH7m34GW,0	Nb, At
D14 <sub>pro</sub> :D14-CERULEAN	Gateway	pH7m34GW,0	At
D14 <sub>pro</sub> :D14 <sup>NLS</sup> -VENUS	Gateway	pH7m34GW,0	At
D14 <sub>pro</sub> :D14 <sup>palm/myr</sup> -CERULEAN	Gateway	pH7m34GW,0	At
35Spro:MAX2-CFP	Gateway	pEarleyGate102	Nb
35Spro:MAX2-CITRINE	Gateway	pB7m34GW,0	Nb
MAX2 <sub>pro</sub> :MAX2-GFP	Conventional	pBI101.3	At

# **Supplemental Table 2**

List of constructs created in this study, with the method of construction and the binary vector used for agrobacterium mediated transformation indicated. The species the construct was transformed into are listed at the right; At = *Arabidopsis thaliana*, Nb = *Nicotiana benthamiana*.

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Clone	Primers
SMXL7 <sub>pro</sub>	<i>F:</i> GGGGACAACTTTGTATAGAAAAGTTGTTTACAGTGTGCGATGTTGAGAC <i>R:</i> GGGGACTGCTTTTTTGTACAAACTTGTCGTCGCCGGTTTAGTTATAA
SMXL7	<i>F:</i> GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGCCGACACCAGTAACCAC <i>R:</i> GGGGACCACTTTGTACAAGAAAGCTGGGTTGATCACTTCGACTCTCGCCG
SMXL6	<i>F</i> : GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGCCGACGCCGGTGACTACG <i>R</i> : GGGGACCACTTTGTACAAGAAAGCTGGGTTCCATATCACATCCACCTTCGCCG
D14 <sub>pro</sub>	F: GGGGACAACTTTGTATAGAAAAGTTGCATTGTCCAGATACAATTC R: GGGGACTGCTTTTTGTACAAACTTGATGTGTTTGGGTTTGAGGGA or Xhol-Notl-F: CTCGAGGCGGCCGCGTCCATTGTCCAGATACAATTCG Kpnl-R: CCCGGGCCGAGGAAGAGCTCGCCGGAGAAC
D14	F: GGGGACAAGTTTGTACAAAAAGCAGGCTTCATGAGTCAACACAACATCT R: GGGGACCACTTTGTACAAGAAAGCTGGGTTCCGAGGAAGAGCTCGCCGGA or KpnI-F: GGTACCATGAGTCAACAACATCTTAG XmaI-R: CCCGGGCCGAGGAAGAGCTCGCCGGAGAAAC
MAX2 <sub>pro</sub>	F: GGGGACAACTTTGTATAGAAAAGTTGGATCCAGGACCATGAGACGT R: GGGGACTGCTTTTTTGTACAAACTTGGAGAAGCGGCAAATCTACAAG or Sall-F: GTCGACCTCATCATGCTTCAACGTGT BamHI-R: GGATCCGAGAAGCGGCAAATCTACAAG
MAX2	F: GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCTTCCACTACTCTCTCCGAC R: GGGGACCACTTTGTACAAGAAAGCTGCGTTGTCAATGATGTTGCGGCTGTTCAA or BamHI-F: CCGTTAGGATCCATGGCTTCCACTACTCTCTC XmaI-R: TAGGTACCCGGGGTCAATGATGTTGCGGCTG
CERULEAN	F: GGGGACAGCTTTCTTGTACAAAGTGGTAATGGTGAGCAAGGGCGAGGA R: GGGGACAACTTTGTATAATAAAGTTGTTTACTTGTACAGCTCGTCCA
CITRINE	F: GGGGACAGCTTTCTTGTACAAAGTGGTAATGGTGAGCAAGGGCGAGGA R: GGGGACAACTTTGTATAATAAAGTTGTCTTGTACAGCTCGTCCATGCC

Supplemental Table 3 Primers used for amplification of promoters, coding sequences and fluorescent protein tags used in this study.