Supplementary File: Materials and Methods

Supplementary Figure Legends

Fig. S1. Transposition efficiency of EP-MS2 insertions in X chromosome. Seven EP-MS2 insertions on the first chromosome were mobilized and the frequency of new insertion events in the autosomes were scored to evaluate the transposition efficiency. The transposition efficiency (Y-axis) is expressed as percentage of red-eyed flies among 300 female progeny (which do not carry the original transgenic first chromosome).

Fig. S2. Crossing scheme for the screen. Following the strategy for EP-expression screens, the EP-MS2 construct on the first chromosome was mobilized by crossing males carrying a transposase-encoding construct (Delta2-3) to females carrying the EP element to be mobilized on the X chromosome. Transposition occurs in the heterozygous progeny. Males from this generation are crossed against *white*$^{-}$females. Individual red-eyed male progeny from this cross are again crossed to *white*$^{-}$ females to establish lines (250 in our pilot screen) for screening. These males have lost the X chromosome carrying the original EP insertion and their red eye color therefore indicates new, transposed insertions on their second or third chromosomes. Males from the next generation of each of thes lines are used for the diagnostic screening crosses. They are mated to females carrying the btl-GAL4, UAS-MS2-GFP chromosome (5 males, 10 females) and the progeny of this cross is analyzed at the third larval instar. At least 16 larvae from each cross are observed under a fluorescent dissecting microscope.

Fig. S3. A-B" High resolution images of terminal cells expressing MCP-GFP and MS2 tagged mRNAs from the negative line EP-MS2-16. MCP-GFP is localized to the nucleus (A-A"). B-B" are blowups of regions marked in A-A" (white box). C-C". In positive lines (here EP-MS2-94) MCP-GFP is seen distributed throughout the terminal cells (C'), including distal branches (D'). D-D" are blowups of regions marked in C-C" (white box). Red signal: auto-fluorescence from the tubes in terminal cells under excitation with 405nm. Scale bars: 10μm.

Fig. S4. Expression levels of ectopically expressed MS2 tagged RNAs.

A Schematic describing the positions of the primer in the inserted EP-MS2 construct (primer Pry2) and the reverse primer from the disrupted gene (gene specific primer). cDNA was synthesized from embryonic total RNA using oligo-dT and random hexamer primers. The forward PCR primer was designed to anneal in the 3' inverted repeat of the EP-MS2 insert (primer JP59/Pry2), the gene specific reverse primers were designed such that when used in combination with primer JP59/Pry2 they would result in amplicons in the range of 300-600bp. The list of gene specific primers is listed in supplementary table S2. B. RT-PCR products from embryo expressing transgenes in their tracheal system Four positive lines (EP-MS2-67: *btsz;* EP-MS2-18: *lola;* EP-MS2-53: *Hr*39; EP-MS2-193: CG30403) and three negative lines (EP-MS2-161: CG13985, EP-MS2-24: CG4455, EP-MS2-3: *bif*) were expressed in trachea together with UAS-GFP using *btl*-Gal4. RT-PCR for *rpl*40 was carried out as an endogenous control, and RT-PCR of Gal4 as a control for the expression of the driver. *Hmg*D was included as a negative control for a gene on the opposite strand of CG30403 (upstream of the direction of transcription). The genes located downstream of the EP insertion sites were all transcribed. CG13895 consistently showed a weaker band than the other genes.

Fig. S5. Validation of candidates.

A-C: Control fluorescent *in situ* hybridization for the experiment shown in Figure 3 with sense RNA probes for *srf*, *dof* and CG9924.

D: Outline of the tracheal cell shown in Figure 3B.

E,F: Terminal branches expressing cytoplasmic GFP (E,F; green in E", F") stained with antibodies against ATPalpha (E) and Dof (F). Both ATPalpha and Dof are found in terminal branches distant from the nucleus.

F: Expression of MS2-GFP in ovaries. Confocal image of two ovarioles, each containing several egg chambers of different stages of oogenesis expressing a UAS-MS2-GFP transgene under the control of nanos-GAL4-V16-GAL4 driver. The same transgenic line was used in our study. For visualisation of GFP, ovaries were dissected, fixed and mounted (no anti-GFP antibody staining was used). GFP is strongly detectable in the nurse cell nuclei and, to a lower extent, in the oocyte nucleus. GFP expression starts in the germarium and is continuous during all stages of oogenesis. Scale bars: E,F=10μm.

Supplementary Table S1: Insertion sites of mapped transposons

Insertion line, cell type	Targeted gene (distance not indicated if at transcription start)	30nt of genomic sequence at insertion site (sequenced from 3’ or 5’ primer)
Trachea		
EP-MS2-12	Within transposable element Doc; many copies in the genome	5’ ATGCATACTTTTCTTTCGTCTCACCGCAAA
EP-MS2-18	lola	3’ GCTCAGTCACCGTCACATTTGCGTCGTTTC
EP-MS2-47	Hsp70Aa	3’ TATTGCTCTCTCACTCTGATCACACAGGAA
EP-MS2-53	hr39	5’ ATGGGGCAGACAGAGAAGAGAGATGGTGAG
EP-MS2-67	btsz, internal	5’ CGCTATTTCTCGTCTTTTGTGCTGCGAAAG 3’ GCAACATCGCCCAGGGAACTGGCTTCGC
EP-MS2-94	btsz, internal	5’ CGCTATTTCTCGTCTTTTGTGCTGCGAAAG
EP-MS2-128	roadkill, intron	5’ ATGAAAACAAAAGTAGTACACGCTGCCGGC
EP-MS2-131	Atp-alpha	5’ AGATGTTACACCGCAGCCTAACGCTCTCAT
EP-MS2-154	no PCR product	
EP-MS2-193	CG30403*	5’ GGAGAGAACTCTCTTACTCTCTCACCAATCC 3’ CGCTAAGCAGGGAAAATGCGTTTTGCACAA
EP-MS2-207	5btsz internal	5’ CGCTATTTCTCGTCTTTTGTGCTGCGAAAG
Neurons		
EP-MS2-16	CG5715	3’ TTCCCCATAAATCAAGAAGCCGGCGGATAG
Oocytes		
EP-MS2-152a	cenG1a	5’ ACTTGGGGGATGATGACGCAAAAGAGAGCA
Negative		
EP-MS2-3	bif, internal	3’ AACCAAACAAAATGGCCAGCACCGACATCT
EP-MS2-9	no gene in correct orientation nearby. cDNA BT022231 8kb downstream	5’ GGCCAAGGAATGTTCAATGACTCAACCAGT
EP-MS2-17	Tre (800bp)	5’ TCGGTTAGTTAAGTTGGCTTAGTTGGCCCAA
EP-MS2-21	Hel89B	5’ GGCCTGACTATCGTGGTGCATCGGTTTTTAG
EP-MS2-24	CG4455	5’ CTGCGGGACATTTTTAATTTATTTCGAATTT 3’ GAACAATCGAAGGGGTTACAAGGCCACGG
EP-MS2-28	UbcD4	5’ TGCATTCGGATAATTCTGAAAGCTCGCCTAT
EP-MS2-51	no gene in correct orientation nearby. CG7879 is 17kb downstream	3’ ATCGAGTGGAACTGGGAGAGAGAGAACCT
EP-MS2-135	CHKov1	5’ ACTCGCAACATATTAGTGGCGTAGTTGTCA
EP-MS2-143	no gene in correct orientation nearby. CG13288 is 6kb downstream	5’ TATTTCATCATGATCGGAAACCACAACACA
EP-MS2-161	CG13895, second intron	3’ TTTGTAAATATACATACATAAATATGTATAA
EP-MS2-187	no gene in correct orientation nearby. dumpy 4kb downstream	5’ CGTTTCACTCAATTTAAGTGACTGCTCACG

Legend supplementary Table S1

The insertion sites of the EP-MS2 vector were mapped by inverse PCR (http://www.fruitfly.org/about/methods/inverse.pcr). The gene listed as targeted gene is the one that is closest to the insertion site in the direction of transcription of the EP-element. The sequence obtained from the flanking regions of insert EP-MS2-12 matched a large number of sites in the genome, which were annotated as ‘Doc natural transposon’, and we were unable to deduce from our sequence data into which of these the P-element had inserted. The PCR reaction with genomic DNA from line EP-MS2-154 did not yield a product, perhaps because the construct was damaged during insertion and had lost at least one of the sites to which the primers anneal. Lines 67, 94 and 207 may be of clonal origin, since their insertion points were precisely at the same nucleotide. Line EP-MS2-152 had two insertions which were subsequently crossed out into two separate stocks. Only the insertion that gave a positive signal in the oocyte is listed here.

* EP-MS2-193 is inserted 1.7kb upstream of the predicted start site of CG30403. However, our RT-PCR data (Fig. S2) show that there is another exon further upstream, in the immediate vicinity of the integration site. RT-PCR between the MS2 motif and a sequence 1.7kb downstream of the integration site within the first *predicted* exon of CG30403 yields a PCR fragment of ca. 450 bp.

Supplementary Table S2: Reagents and stocks

Primers used for RT-PCR experiments		
btsz	btszCT-F	GAGTTCGCCATGCAGTACAA
	btszCT-R	TCGAGGAGCTGCCTGTTATT
CG30403	JP79	GTCTCCGATCTTGGGCACGG
	JP81	CAGTCCGGGATCAATGCTGA
*hr*39	JP84	ATTCTCGCCATTGGACGAGAG
	JP86	GAAATCATGGATGTAGAGCA
EP-MS2-67	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
btsz	JP40	CTGGTCGGGTCTTTGTGTCT
EP-MS2-18	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
lola	JP45	AGCACTGCAAAGGTTGGTTT
EP-MS2-53	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
*Hr*39	JP46	GTTCGGTGACGGTAGTTCGT
EP-MS2-193	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
CG30403	JP75	CTGAATTCGCCGATAGGC
EP-MS2-161	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
CG13985	JP54	CGCTGTGGAGGTAAAACTGG
EP-MS2-24	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
CG4455	JP56	GAGGCAGAGAACGATTACGG
EP-MS2-3	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
bif	JP48	TGCAGCTTTTGCTGTTATCG
EP-MS2-193	JP59/Pry2	CTTGCCGACGGGACCACCTTATGTTATT
*Hmg*D	JP42	TAGTCGTCCTTGGCCTTAGC
	JP67	GGTCTTCGAGTCAGGTTCCA
Gal4	JP68	CGTCGACAGAAGAAGGGAAG
	RPL49-for	TCCTACCAGCTTCAAGATGACC
Rpl49	RPL49-rev	CACGTTGTGCACCAGGAACT

Primers used for cloning	
eGFP primer1	GCAGCGGCCGCAGTGAGCAAGGGCGAGGAGC
eGFP primer2	CGTCTAGATTACTTGTACAGCTCGTCCATGCC

Fly Stocks	**Source**
P{w[+mC]=*btl*-Gal4}	Ref (1)
nanos-GAL4::VP16	Ref (2)
$GAL4^{4\text{-}77}$, UAS-mCD8::GFP	Ref (3)
UAS-MCP-RFP	Ref (4)
UAS-Fng-ADD-myc	Ref (5)
UAS-KDEL-GFP	Bloomington, 9899
*Hsp*70Aa-RNAi	VDRC, 31366
lola-RNAi	VDRC, 12052

CG9924-RNAi	VDRC, 28798
CG30403-RNAi	VDRC, 19223
ATPalpha-RNAi	VDRC, 12330
btsz-RNAi	VDRC, 102608
*Hr*39-RNAi	VDRC, 37695
Antibodies and other reagents	**Source**
rabbit anti-Pac (1:500)	S. F. Newbury; ref (6)
rabbit anti-Dof (1:200)	Ref (1)
rabbit anti-cMyc (1:100)	Santa Cruz Biotechnology
rabbit anti-GFP (1:500)	Torrey Pines Biolabs Inc
rabbit anti-Dcp1 (1:200)	Tze-Bin Chou; ref (7)
anti-mouse BicD (1:200)	DSHB
mouse anti-ATPalpha (1:100)	DSHB
Alexa- secondary antibodies (1:500)	Invitrogen
DAPI and Hoechst	Sigma
Alexa488-TSA (TSA kit # 22, T2 0932)	Invitrogen

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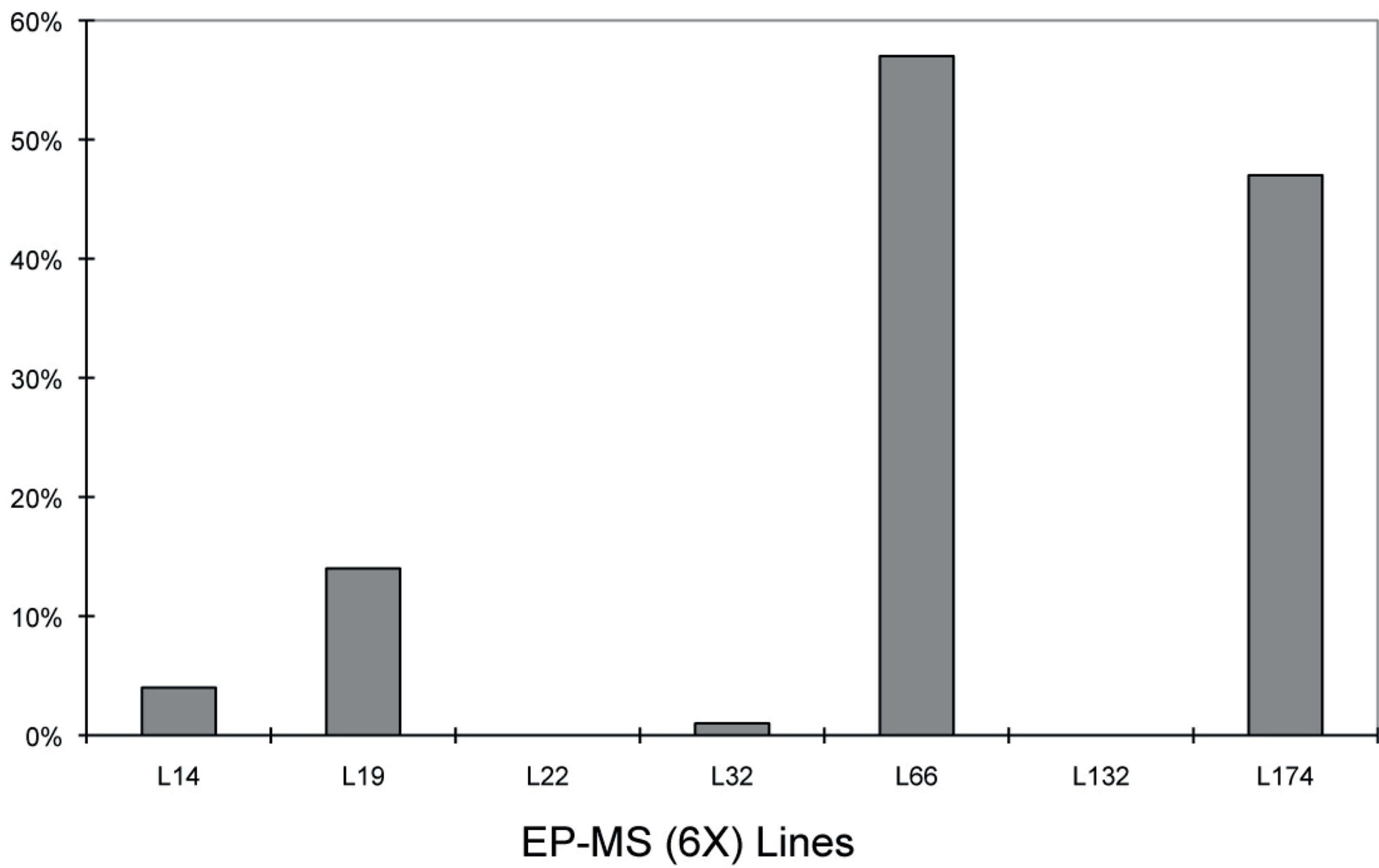
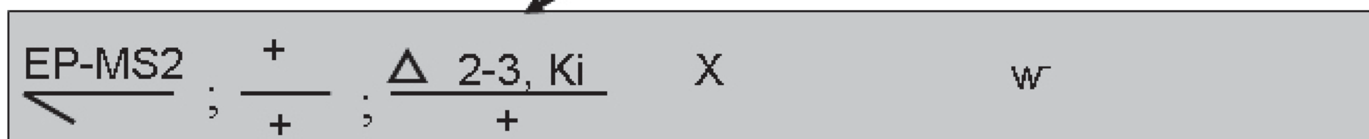
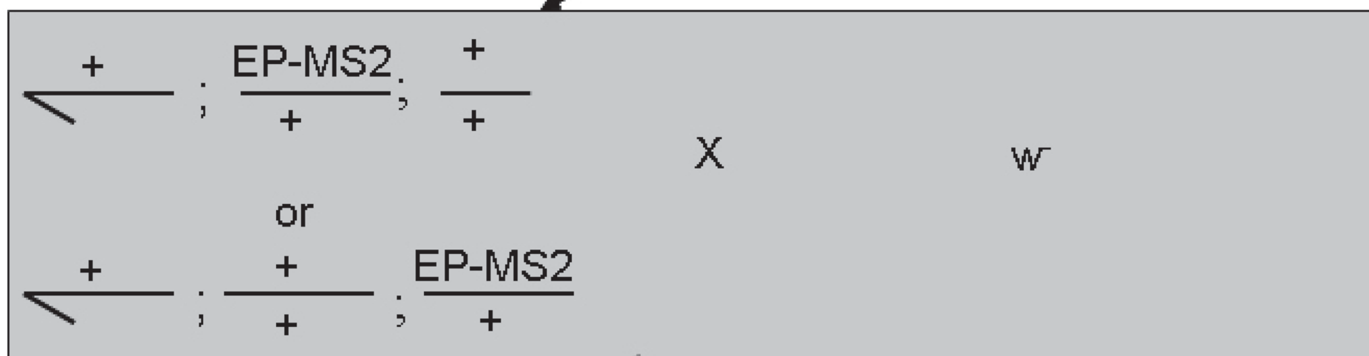


Figure S1

A





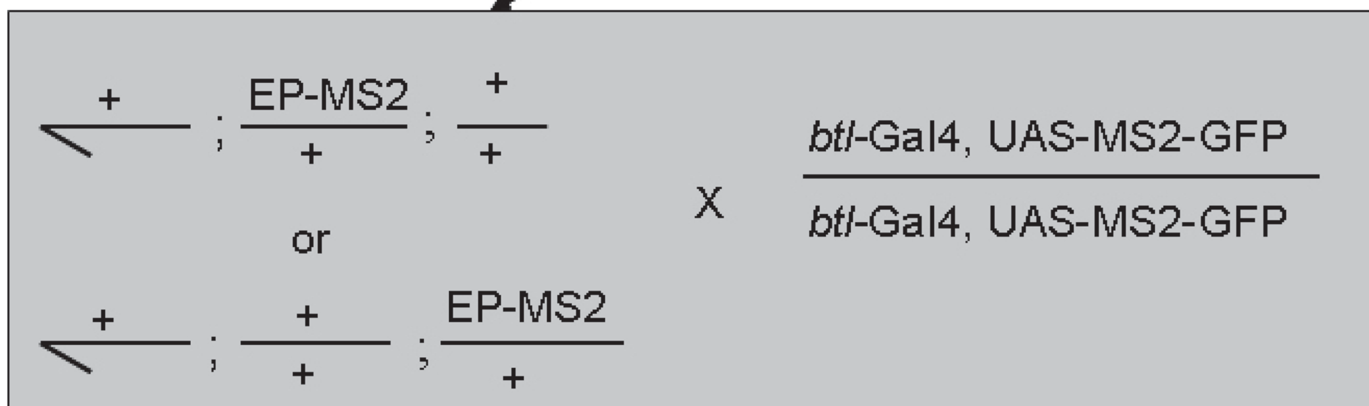


Figure S2

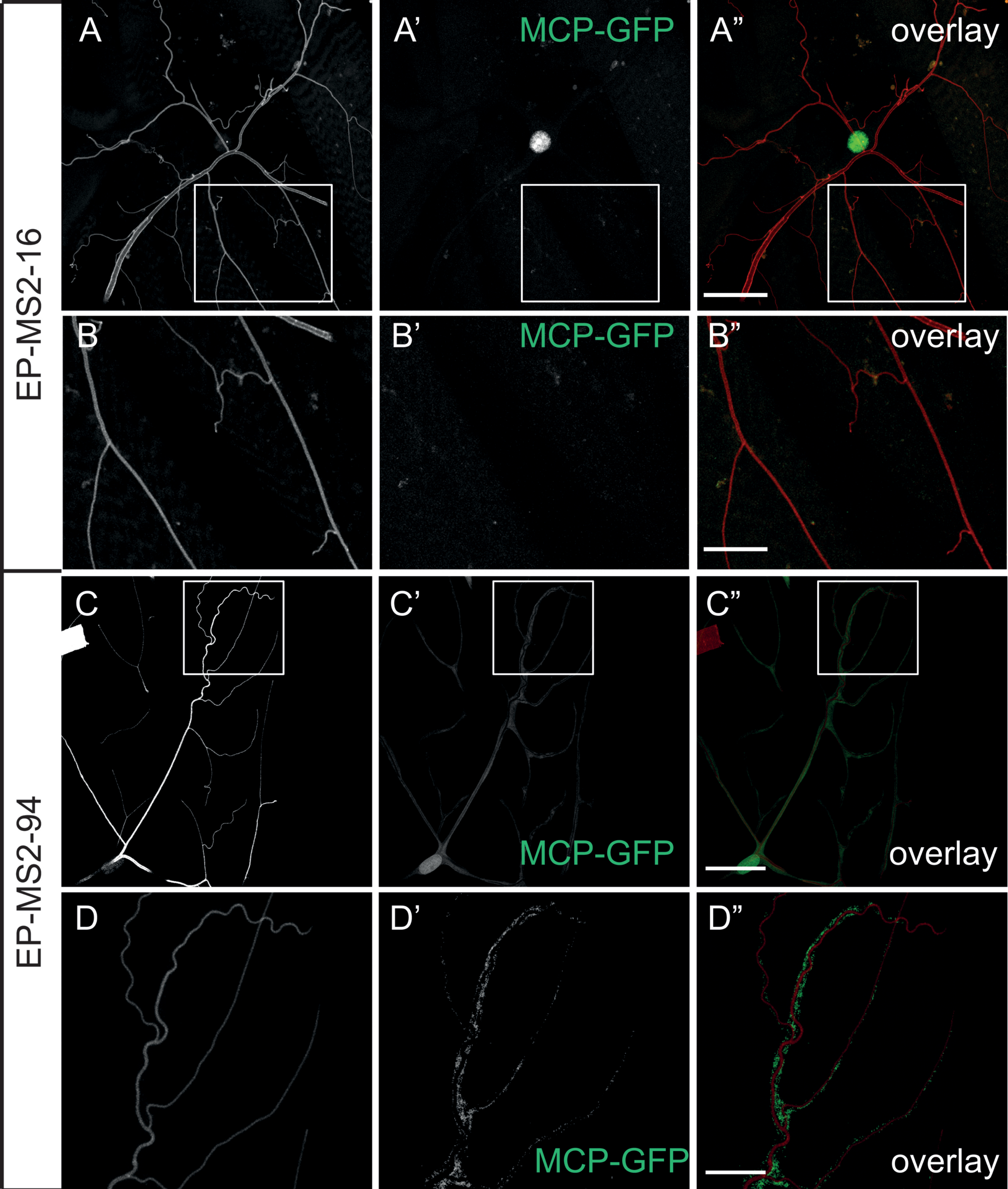
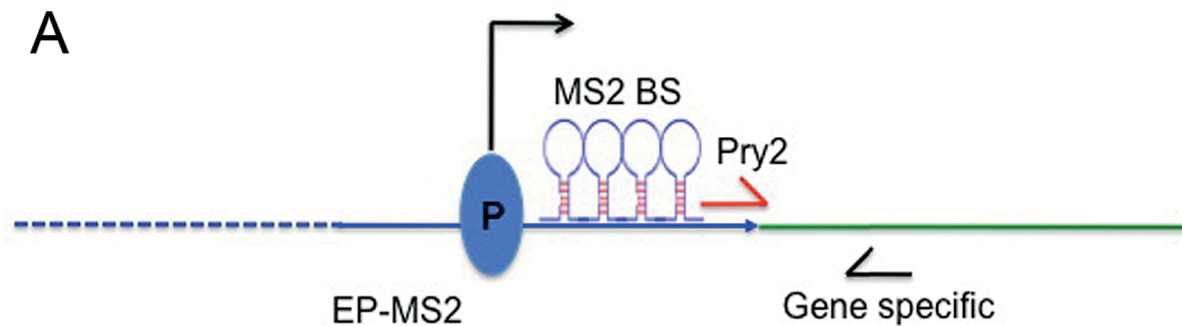


Figure S3

A



B

Gene specific | *rpl49* | *Gal4* | | Gene specific | *rpl49* | *Gal4*

btsz
lola
*hr*39
CG30403

CG13985
CG4455
bif
*Hmg*D

Figure S4

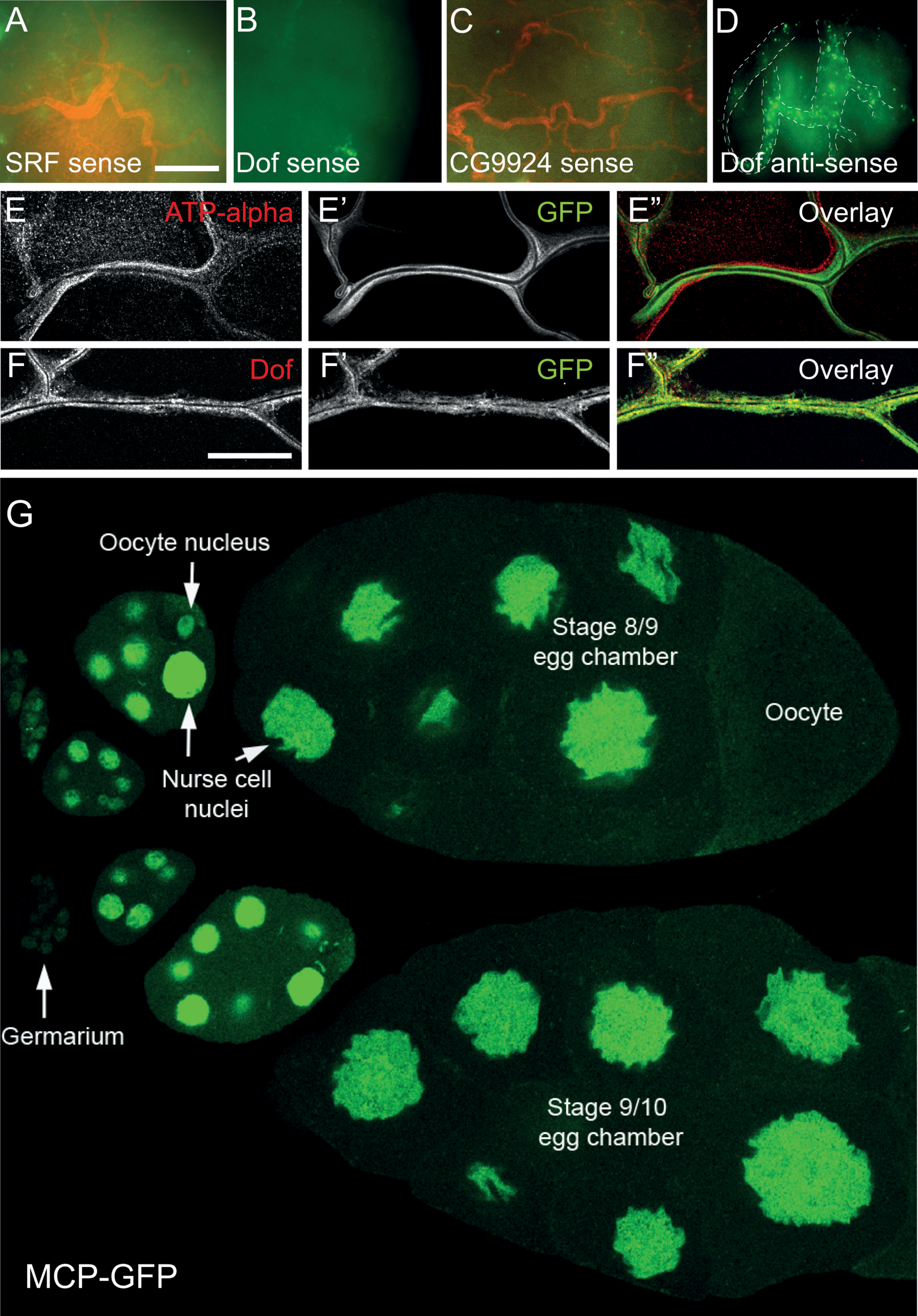


Figure S5