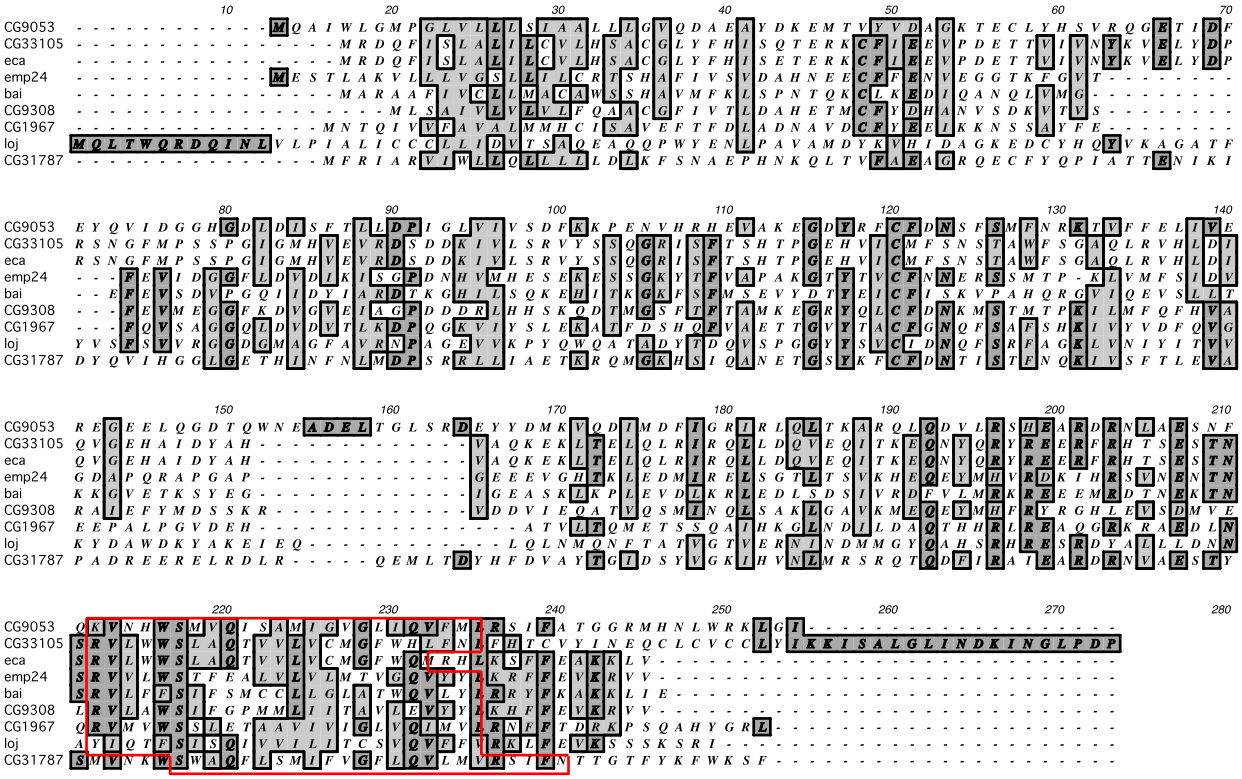
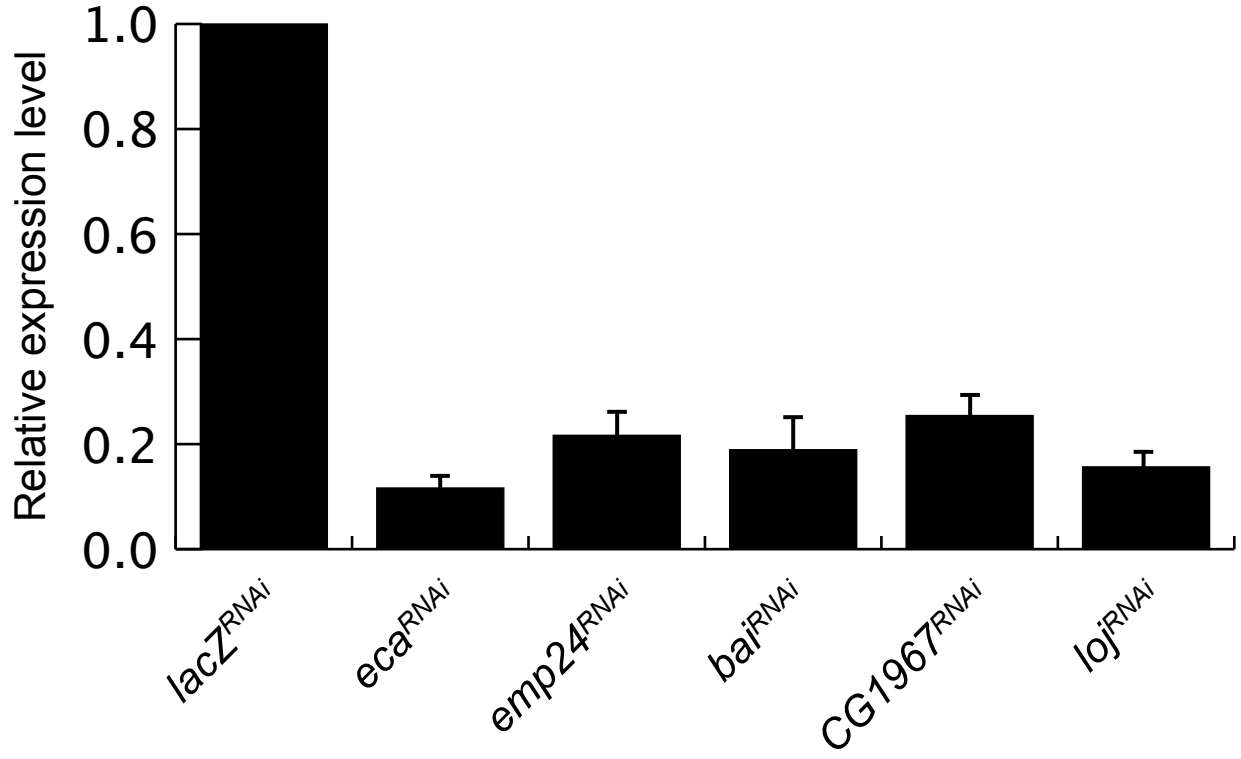
Supplementary material for: Port F , Hausmann G, Basler K:

A genome-wide RNA interference screen uncovers two p24 proteins as regulators of Wingless secretion

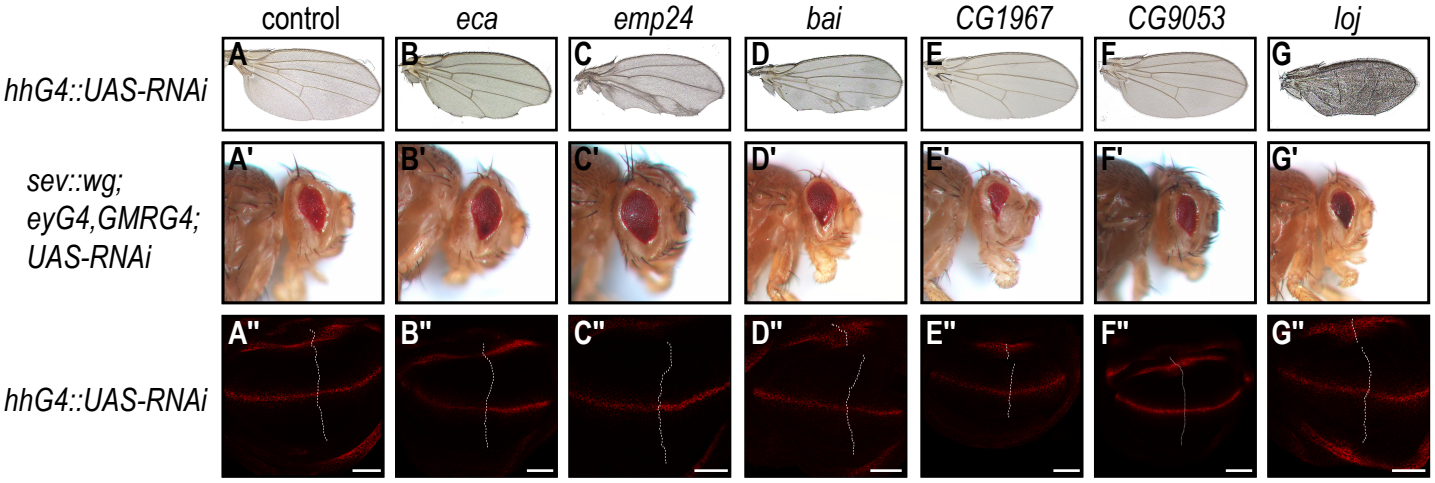


Supplementary Figure 1: The p24 protein family in *Drosophila melanogaster*. Alignment of the nine predicted p24 proteins in *Drosophila*, performed with MacVector software. Transmembrane domains as predicted by TMHMM (www.cbs.dtu.dk/services/TMHMM) are boxed in red. Note that *CG33105* most likely arose from a gene duplication event from *eca*. The protein encoded by *CG33105* is identical to Eca except for the extended C-terminal tail, which is untypical for p24 proteins. We found no evidence for expression of *CG33105*.

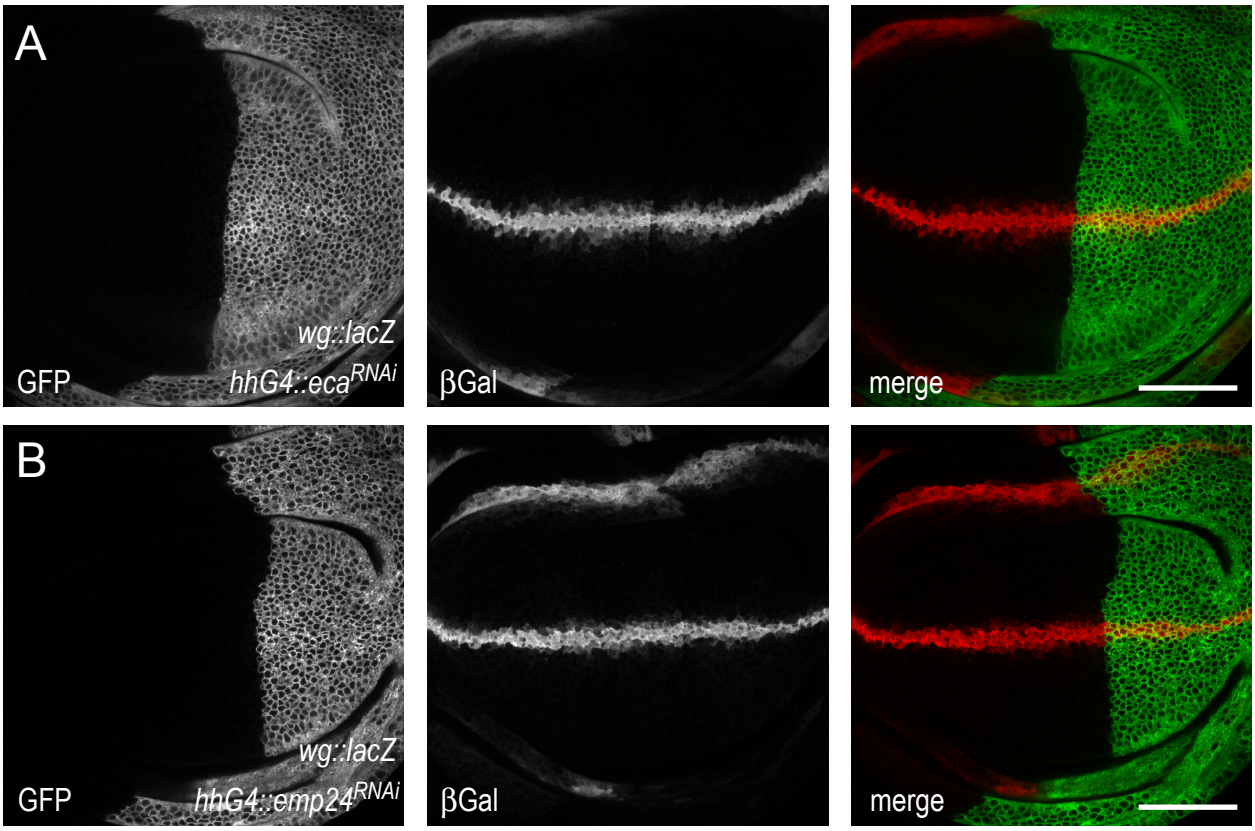


Supplementary Figure 2: Efficient knock-down of mRNA levels by RNAi. Imaginal discs from third instar larvae were analysed by semi-qt-RT-PCR. Total RNA was extracted from approximately 20 wing imaginal discs from each genotype using the Nucleospin RNA II kit (Macherey-Nagel). Semi-quantitative PCR reactions were performed in triplicates and monitored using the Applied Biosystems SYBR Green kit and the ABI Prism 7900HT System (Applied Biosystems). All results were simultaneously normalized to the Actin5C, tubulin-1a and TBP mRNA levels and the expression levels calculated using the DDCt method (Applied Biosystems user bulletin #2, updated version 04/2001). Relative change of mRNA abundance relative to control imaginal discs (UAS-lacZ^{RNAi}) is shown. Error bars represent standard deviation. Readings for CG9053^{RNAi} were highly variable and are not shown. We used the following fly lines from the Vienna *Drosophila* RNAi Center: eca 101388, emp24 100274, CG1967 100594. We generated RNAi lines for bai and loj according to the method described by Haley et al. (Haley et al., Dev Biol, 2008) using the following oligos:

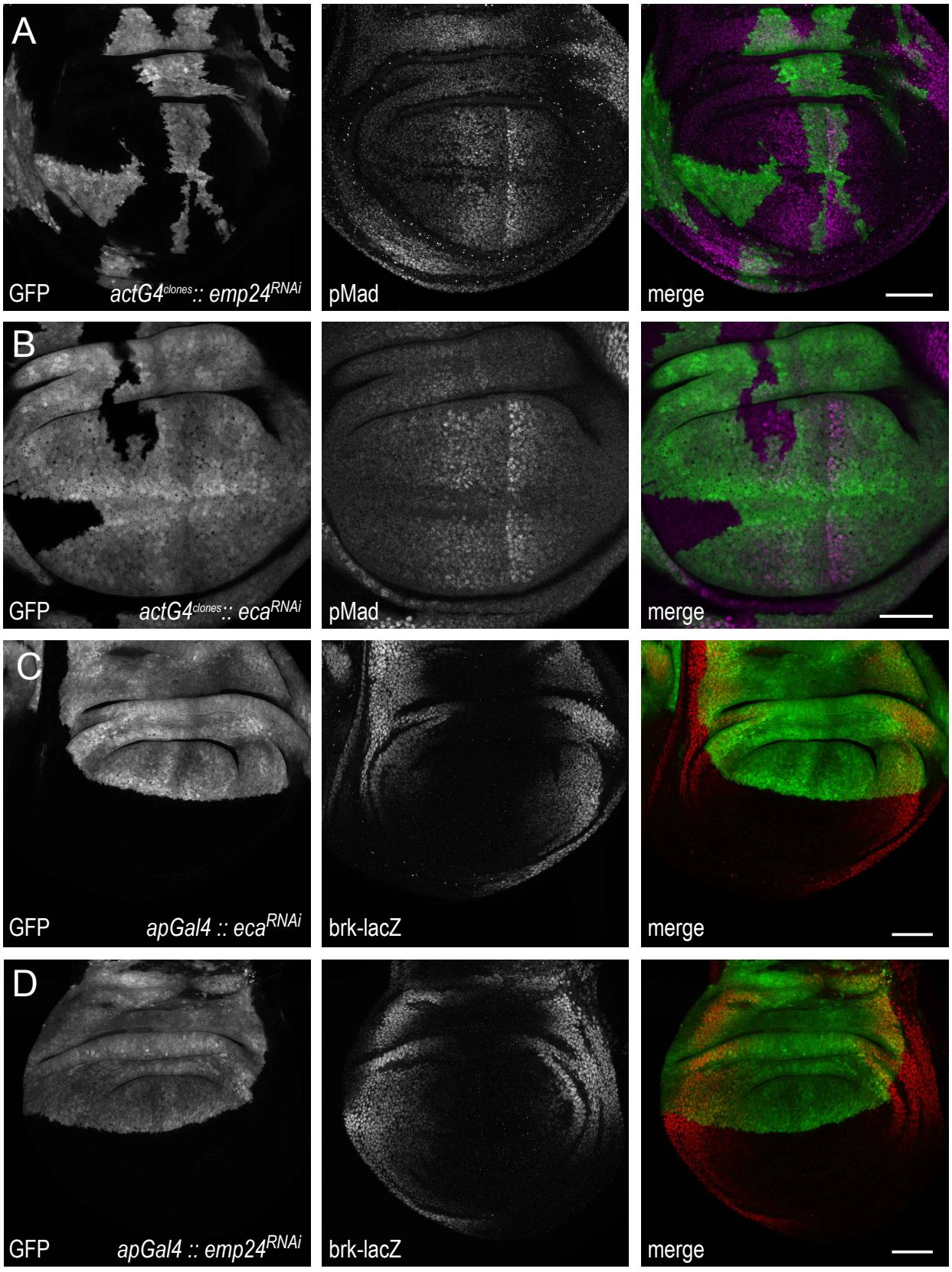
bai top *ctagcagtGCGTGCGTGGTACTGTGTTAAtagttatattcaagcataTAAACACAGTTCCACGCACGCgcg*
bai bottom *aattcgcGCGTGCGTGGAACTGTGTTTAtatgcttgaatataactaTTAACACAGTACCACGCACGCactg*
loj top *ctagcagtACTTATGTCAAACGAGTTAAAtagttatattcaagcataTATAACTCGTATGACATAAGTgcg*
loj bottom *aattcgcACTTATGTCATACGAGTTATAtatgcttgaatataactaTTTAACTCGTTTGACATAAGTactg*



Supplementary Figure 3: Knock-down of p24 genes in *Drosophila* by RNAi. RNAi hairpin transgenes were expressed with the Gal4-UAS system using the Gal4 drivers indicated on the left. *hhGal4* drives expression in the posterior compartment of the wing imaginal disc (right to the dashed line in A'' - G''), which gives rise to the posterior of the adult wing (lower halve of the wings in A - G). We used a hairpin designed to target the *lacZ* gene as a negative control (A - A''). Knock-down of *eca* or *emp24* gives rise to wing notches and partially suppress the rough-eye phenotype caused by overexpression of Wg (B, B', C, C'). Importantly, eca and emp24 RNAi also causes accumulation of endogenous Wg in producing cells (B'' and C'' and Figure 3). Knock-down of *emp24* also induces wing vein defects that are not typically associated with Wg signaling defects. Knock-down of *bai* and *loj* do cause wing phenotypes including wing notches, but do not suppress the Wg overexpression phenotype in the eye and do not affect trafficking of endogenous Wg protein (D - D'' and G - G''). Knock-down of *CG1967* and *CG9053* does not cause any phenotype. Scale bar reprensents 50µm.



Supplementary Figure 4. Eca or Emp24 are not required for Wg transcription. Knock-down of either *eca* or *emp24* does not affect *wg* transcription, as revealed by normal expression of *wg-lacZ*. The RNAi hairpin constructs are expressed in the posterior compartment, that is marked by co-expression of CD8-GFP. Scale bars represent 50μm.



Supplementary Figure 5: Eca or Emp24 are not required for Dpp signaling in wing imaginal discs. RNAi hairpin constructs were expressed in clones of cells using actinGal4 (**A** and **B**) or in the dorsal compartment under the control of apterous-Gal4 (**C** and **D**) (compartments marked by GFP expression). Phosphorylation of Mad protein was detected with an specific antibody and no change of phosphorylation was observed (**A** and **B**). Brinker expression was monitored by means of a brk-lacZ line and no difference in *brinker* expression was observed between the dorsal and ventral compartment of the disc (**C** and **D**).