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Epithelial monitoring via ligand-receptor segregation ensures malignant cell elimination

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Abstract

Animals have evolved mechanisms, such as cell competition, to remove dangerous or nonfunctional cells from a tissue. Tumor Necrosis Factor signaling can eliminate clonal malignancies from *Drosophila* imaginal epithelia, but why this pathway is activated in tumor cells, but not normal tissue, is unknown. We show that the ligand driving elimination is present in basolateral circulation, but remains latent because it is spatially segregated from its apically-localized receptor. Polarity defects associated with malignant transformation cause receptor mislocalization, allowing ligand binding and subsequent apoptotic signaling. This process occurs irrespective of the neighboring cells' genotype and is thus distinct from cell competition. Related phenomena at epithelial wound sites are required for efficient repair. This mechanism of polarized compartmentalization of ligand and receptor can generally monitor epithelial integrity to promote tissue homeostasis.

One sentence summary:

Transformed cells and wounds activate a polarity-enforced latent signaling system to trigger death or repair respectively.

Epithelial architecture is the fundamental organizing principle of animal tissues. Polarized epithelial sheets provide a contiguous barrier that allows an organ to function in a milieu distinct from the external environment. To maintain the barrier, epithelia must detect threats to their integrity and resolve them. Integrity can be compromised by both physical damage and the production of structurally-defective cells. The latter is a frequent feature of oncogenic transformation, and it is important to eliminate such cells before a tumor can form. Deleterious cells can be removed by cell competition, a broadly utilized mechanism in which 'winner' cells of one genotype often induce apoptosis in neighboring 'loser' cells (1, 2). In *Drosophila* imaginal discs, cells mutant for the conserved apicobasal polarity regulators *scribble* (*scrib*) or *discs-large* (*dlg*) form malignant, 'neoplastic' tumors that kill

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the animal (3, 4). However, prior to tumor growth, small clones of these polarity-deficient cells are efficiently eliminated, allowing a healthy organ to develop. The mechanisms involved have been described as cell competition during which the *Drosophila* TNF ligand Eiger (Egr) binds the TNF receptor Grindelwald (TNFR, Grnd) in mutant cells, activating the JNK Basket (Bsk), which induces apoptosis (reviewed in (5–7)). How polarity loss is coupled to TNF pathway activation to remove oncogenic clones is not known.

We investigated TNF-TNFR interactions during polarity-deficient cell elimination by co-culturing imaginal discs *ex vivo* alongside Egr-Venus (EgrV)-expressing fat bodies (a major endocrine organ) (Fig. 1A)(8). EgrV secreted into media associated strongly with clones of *dlg*-depleted cells (Fig. 1B–C, E, Fig. S1A–B, K–L). Increased EgrV binding is specific for polarity-deficient elimination: it is seen in *scrib* mutant clones but not loser cells outcompeted by Myc-overexpressing neighbors (Fig. S1G–J). Egr binding, like cell elimination, depends on Grnd (Fig. 1D–F, Fig. 2I, Fig. S1C, Fig S3C–E). We used *patched-GAL4 (ptc-GAL4)* to conditionally deplete *dlg*, generating a consistent stripe of apoptotic cells that accumulate EgrV (Fig. 1G–L, Fig. S1D–E, Fig. S3A–B)(9, 10). As in clones, Grnd depletion blocked elimination and led to overgrowth (Fig. 1K, Fig. S1F). Mechanical wounding also activates JNK signaling (11), and wound sites bind secreted Egr in a Grnd-dependent manner (Fig. S2A–I). Increased Egr-Grnd binding is thus associated with malignant cell elimination and physical wounding, which both disrupt epithelial integrity.

Since JNK activation in both cases above is associated with Egr binding, we investigated the underlying mechanism. Data argue against elevated Grnd levels (Fig. S5A–R), changes in Grnd N-glycosylation (8)(Fig. S5S), altered endocytic dynamics (12)(Fig. S4A–L), or elevated Egr levels (Fig. S6A–Q) as mediators of *dlg*-depleted cell apoptosis. In functional experiments, neither autocrine nor paracrine epithelial Egr was required (Fig. 2A, D–F, H, L, Fig. S3F, J). Because polarity-deficient clones survive in an animal completely devoid of Egr (Fig. 2G, L)(10, 12, 13), the Egr required for elimination must come from another source.

Both fat body and hemocytes (innate immune cells) produce Egr (14–17). We co-depleted *egr* and *dlg* simultaneously from both the disc stripe and these tissues. Hemocytes did not associate with Dlg-deficient cells, and co-depletion of hemocyte Egr had no impact on elimination (Fig. 2B, D, Fig. S3G, Fig. S6R, S). Co-depletion of fat body Egr prevented apoptosis in the stripe: Dlg-deficient cells persisted and overgrew (Fig. 2C–D, Fig. S3H–J). *scrib* mutant disc clones persisted upon Egr depletion in fat body and hemocytes (Fig. 2J, L), but not when Egr was depleted in hemocytes alone (Fig. 2K–L). Wound healing was also perturbed by depletion of fat body Egr (Fig. S2J–K). Together, these data indicate that circulating Egr is essential for full activation of Grnd and JNK signaling in response to epithelial interruptions.

The above results prompt consideration of ligand and receptor localization in this signaling axis. Fat body-produced Egr is secreted into hemolymph (circulatory fluid), which bathes the disc basolateral surface (Fig. 3A)(15). However, steady-state Grnd is apically polarized (Fig. 3B, C)(7). Co-culture experiments revealed that EgrV binds only basally, suggesting limited access of circulating Egr to Grnd (Fig. 3C, Fig S8D–E). Dextran assays demonstrated that discs do not display transepithelial permeability, but luminal access can

be induced by wounding (Fig. 3D–E). We tested whether fat body-produced EgrV could bind to an extracellular nanobody targeted to either polarized epithelial surface *in vivo* (Fig. S8A)(18). EgrV bound robustly to basal nanobodies (Fig. 3F) but only slightly to apical nanobodies, and basal signal of these cells was higher (Fig. 3G). After mechanical wounding of the latter discs, EgrV bound apically instead (Fig. 3H). Enhanced EgrV binding was also seen when Dlg-depleted cells mispolarize apical nanobodies (Fig. S8B–C). Thus, TNF ligand and its receptor are normally segregated by the epithelial barrier. However, inducing transepithelial permeability was not sufficient to initiate cell elimination (Fig. S7A–J).

We therefore examined Grnd localization during polarity-deficient cell elimination and found it mispolarized basolaterally (Fig. 4A–B, Fig. 3C, Fig. S8F–G). This is not due to cell death or basal extrusion (Fig. S5P–R). When these discs are co-cultured, bound EgrV is predominantly basal (Fig. 4A–B). Inhibiting JNK rescued apoptosis but not basal Grnd localization, and again EgrV bound basally (Fig. 4C, Fig. S8H). In wounded cells also, EgrV bound predominantly basally, although tissue damage prevented rigorous analysis of Grnd localization (Fig. 4D, Fig. S8I). These data suggest that receptor mispolarization allows access to basally circulating ligand, triggering JNK activation, and adaptive homeostatic responses including cell elimination and wound-healing.

Elimination of polarity-deficient cells has long been described as a form of cell competition, albeit regulated by pathways distinct from *Minute* or *Myc* competition (1, 2, 5–7). A defining feature of cell competition is that elimination of ‘loser’ cells requires neighboring ‘winners’ of a different genotype. Yet circulating Egr can access basolaterally-mislocalized Grnd in any polarity-deficient cell, regardless of its neighbor. We therefore reconsidered the requirement for WT cells in death of *scrib*-class mutant cells.

We asked whether polarity-deficient discs containing no WT cells showed the same dependence on circulating Egr as polarity-deficient cells with WT neighbors. *scrib* discs bound EgrV specifically at their hemolymph-contacting periphery, paralleling the activation of JNK reporters (Fig. 4E–F, Fig. S9A–B)(19). Apoptosis in *scrib* discs is also enriched peripherally, compared to the ‘core’ which lacks EgrV binding (Fig. 4J). Peripheral apoptosis and JNK activation were normalized when circulating Egr was depleted, and *scrib* discs were larger, consistent with a hypothesis that multilayered tissue architecture allows some *scrib* cells to evade hemolymph Egr and overproliferate to form tumors (Fig. 4F–L)(19). *scrib* disc periphery mitotic rates were elevated in fat body Egr-depleted animals, likely due to relief of JNK-mediated cell cycle stalling (Fig. S9C–E) (20). Thus, the same mechanisms that eliminate small clones of polarity-deficient cells also kill polarity-deficient cells and limit their growth in a non-competitive situation. These results challenge the paradigm that elimination of *scrib* cells is due to classical cell competition, and suggest that the mechanism we describe is a distinct pathway coupling epithelial organization to tissue homeostasis.

All epithelia need to monitor their integrity and respond when breaches are detected. Since most tumors arise in epithelial tissues, preventing the growth of malignant clones within them must also be a priority. Here we show a mechanism for tumor elimination that arises from an intrinsic property of the epithelial barrier –its ability to compartmentalize a luminal

environment segregated from the external milieu. *Drosophila* TNF circulates systemically and bathes basal organ surfaces, but is latent due to TNFR's strict apical localization. However, when neoplastic cells arise, their altered polarity induces basal localization of TNFR, where it binds ligand and triggers apoptotic signaling. A similar axis promotes wound healing: if the epithelium is physically ruptured, ligand can meet receptor and contribute to a pro-healing JNK signaling program. Thus, a common molecular mechanism inherent to epithelial geometry -a mechanism which recognizes polarity changes as a Damage-Associated Molecular Pattern (DAMP)(21) -- underlies both homeostatic programs (Fig. 4M). The function of the TNF/TNFR system described here as an *in vivo* sensor of epithelial integrity raises the possibility that ligand-receptor segregation (22) may be a theme general to epithelial maintenance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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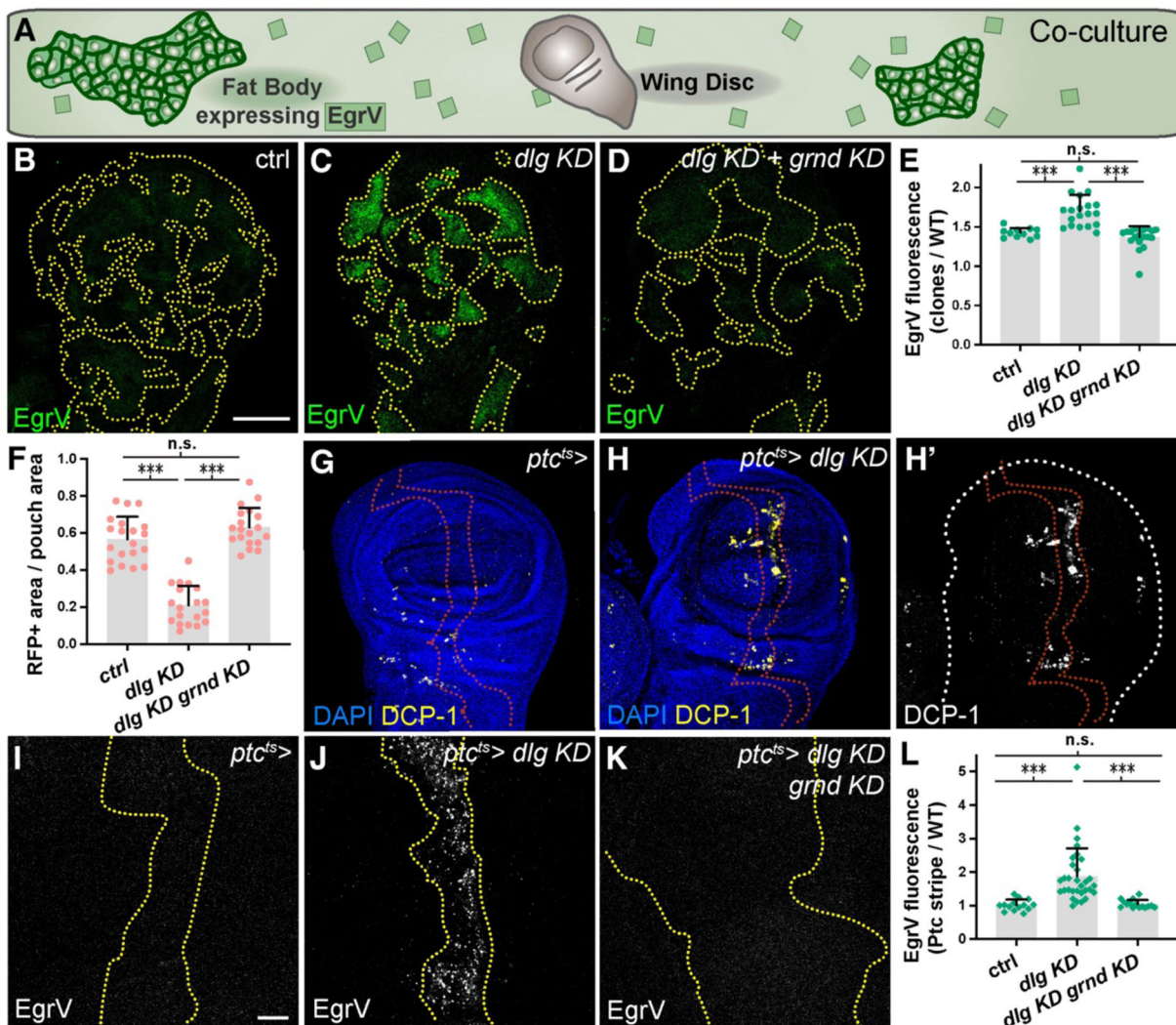


Fig. 1. Heightened binding of Egr to polarity-deficient cells

(A): Diagram of EgrV-expressing fat bodies co-cultured *ex vivo* with imaginal discs.

(B-F): Binding of EgrV and elimination of *dlg*-depleted clones **(C)** are dependent on cell-autonomous Grnd **(D, control in B, dotted lines demarcate clones)**. Quantitated in **E, F**.

(G-H): Depletion of *dlg* with *ptc-Gal4* (dotted lines indicate stripe of expression) enhances cell death (DCP-1) **(H, control in G, quantitation in Fig. 2D)**

(I-L): EgrV binding, absent in control **(I)**, is elevated in the *dlg*-depleted stripe **(J)**, and this requires Grnd **(K)**. Quantitated in **L**.

Scale bars: 100 μ m in **B**, 10 μ m in **I**. See Materials and Methods and Table S3 for information on statistical tests.

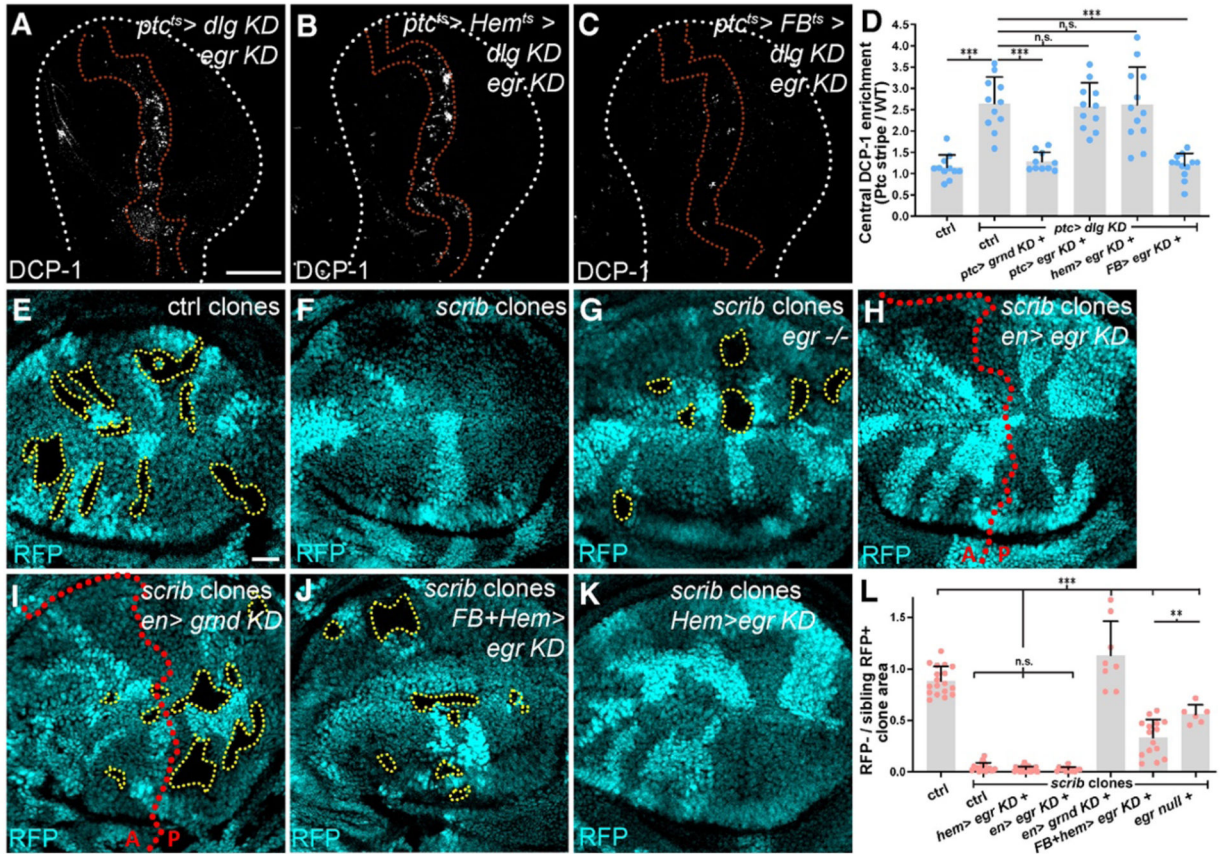


Fig. 2. Egr required for cell elimination derives from circulation

(A-D): *dlg* cell elimination in the stripe (DCP-1, red dotted lines) is not prevented by co-depletion of autonomous *egr* (A), nor by *egr* co-depletion in hemocytes (B). Depletion of *egr* from fat body and stripe prevents *dlg* cell apoptosis (C). Quantitation of apoptosis shows requirement for autonomous *Grnd* and fat body-produced *Egr* (D).

(E-L): Although WT mitotic clones survive (E), *scrib* clones are eliminated (F). *scrib* clones survive in entirely *egr*-mutant animal (G), but are eliminated in a field of *egr*-depleted cells (H). As with autonomous depletion of *grnd* (I), *scrib* clone elimination is blocked when *egr* is depleted in fat body and hemocytes (J), but not in hemocytes alone (K). Quantitation in L. Dotted lines mark clone boundaries or posterior (P) compartment gene depletion.

Scale bars: 100 μ m in A, 25 μ m in E.

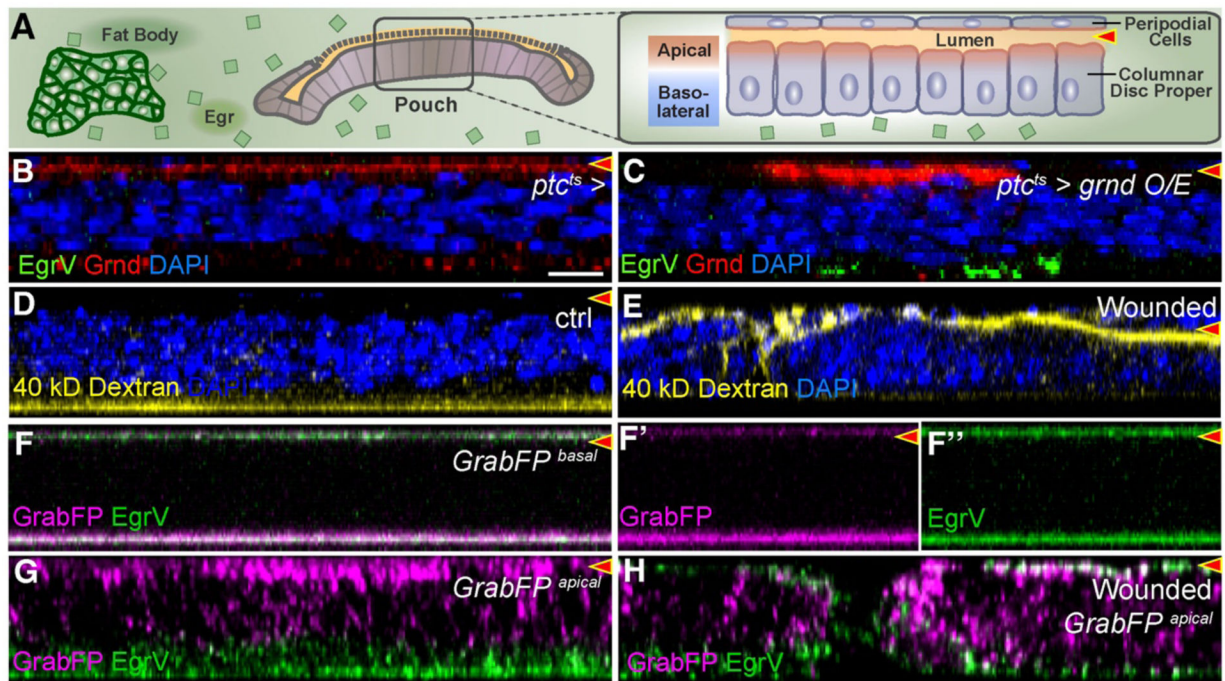


Fig. 3. Egr binds basolaterally to polarity-deficient cells

X-Z cross-sections show disc proper below and peripodium above. Lumen is indicated by red arrowhead.

(A): Diagram showing relationship of disc epithelial barrier to hemolymph. Egr secreted by fat body bathes the basolateral surface but is excluded from apical surface and lumen.

(B-C): Grnd is apically localized **(B)**, even when overexpressed **(C)**, but bound EgrV is exclusively basolateral.

(D-E): Dextran in media is excluded from lumen of intact discs **(D)** but can enter wounded discs **(E)**.

(F): Basolateral GrabFP binds strongly at basal surface to EgrV produced by fat bodies. Signal at top is peripodial basal surface. **F'** and **F''** show left half of **F**.

(G-H): Apical GrabFP binds EgrV only at the basolateral surface **(G)**, but wounding enables strong apical binding of EgrV as well **(H)**.

Scale bar: 10 μm in **B**

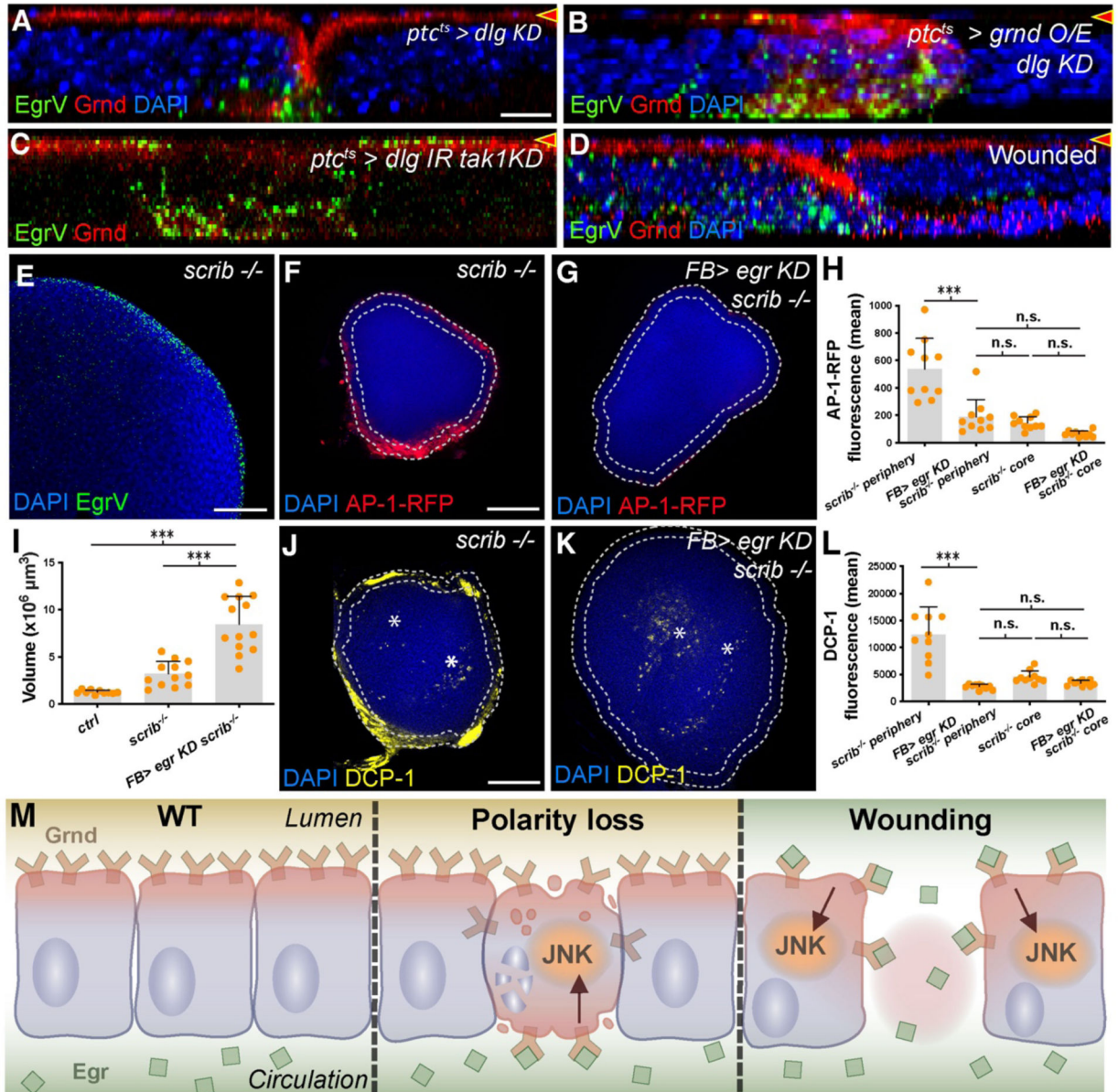


Fig. 4. Mispolarization of Grnd permits Egr binding and cell elimination

(A-D): X-Z sections as in Fig. 3. *dlg*-depleted cells mislocalize Grnd basolaterally (A), evident especially when Grnd is overexpressed (B). EgrV binds basally to *dlg*-depleted cells. Co-depletion of *dlg* and *tak1* (C) blocks cell elimination but polarity defects remain. Mispolarized Grnd binds EgrV at basal surface. Wounded discs (D) show altered Grnd localization and basal EgrV binding.

(E-L): X-Y sections. Wing discs containing only *scrib* mutant cells bind Egr preferentially at periphery (E). JNK signaling (F) is elevated in periphery compared to core and is dependent on circulating Egr (G, quantitated in H). *scrib* discs grow larger when circulating Egr is depleted (I), and peripheral apoptosis (J, DCP-1) is reduced (K, asterisks indicate DCP-1+ cells in core; quantitated in L).

(M): Model for role of polarized segregation of TNF ligand and receptor in epithelial homeostasis.

Scale bar: 10 μm in **A**, 50 μm in **E**, 100 μm in **F**, and **J**.

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