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# D52 Molecule Page

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**Gene Symbol: TPD52**

## 1. General description structure and expression of D52

### Discovery of D52

Tumor Protein D52 (*TPD52, D52*, also referred to as *PrLZ* or *PC-1*) is the founding and most highly studied member of a small mammalian gene family that also includes *D53/ TPD52L1, D54/ TPD52L2,* and *D55/ TPD52L*. D52 was first identified in $P^{32}$-orthophosphate labeled pancreatic acinar cells as a calcium regulated heat stable phosphoprotein of 28 kDa (CRHSP-28) by two-dimensional electrophoresis (8). While that study was under review, Parenta et al. reported the same phosphoprotein in isolated gastric mucosa cells and named it calcium sensitive phosphoprotein of 28 kDa (CSPP28) (17). Purification and sequencing by Edman degradation indicated these molecules were identical to the predicted open reading frame of an mRNA termed Tumor Protein D52. The D52 mRNA was first discovered in 1995 by Byrne et al. through a screen for genes commonly overexpressed in invasive breast cancer and basal cell carcinomas (2, 4). D52 was also independently identified in proliferating cells and given the names *N8* and *R10*. *N8* was discovered as a novel gene differentially expressed in lung cancer versus fibroblast cell lines, whereas the *R10* gene was found to be highly expressed in proliferating avian embryonic neuroretinal cells following retroviral infection (5, 18).

### D52 protein structure

D52 proteins are highly conserved mainly within a coiled-coil region from lower metazoans to humans but bear little homology to other proteins (1, 7). Human D52 is a small (184 residues) acidic protein that runs anomalously at 25-28 kDa on SDS-PAGE. Highly charged, the overall pI of D52 is 4.75 with a basic central region (aa 50-125) that is flanked by acidic *N- and C-termini* (**Fig 1**). The

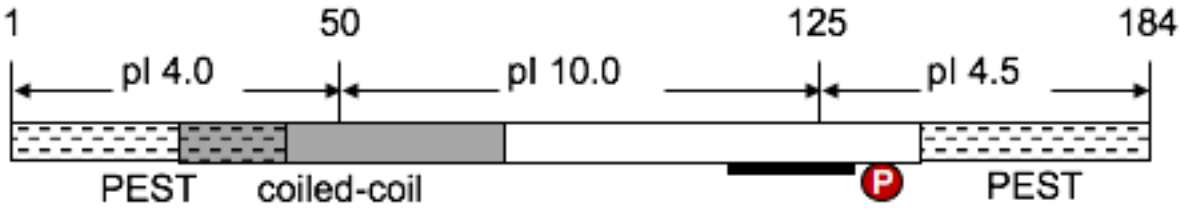


**Figure 1:** A schematic of the D52 protein indicating the acidic *N- and C-termini*. PEST sequences are denoted by the dashed lines and the coiled-coil region is in grey. The predicted (Ser111-131) interaction region is indicated by the black bar and the confirmed (Ser136) phosphorylation site is marked with a red circle.

single distinguishing structural feature of D52 is a coiled-coil domain from Glu27-Leu71 that facilitates both homo- and heteromeric interactions with other TPD52 family members (1). D52 undergoes calcium-stimulated phosphorylation at Ser136 but is not regulated by other G-protein coupled acinar cell signaling molecules (diacylglycerol, cAMP, cGMP, growth and stress kinases) (11). D52 has been shown to undergo degradation by lysosomal cathepsins in acini and also contains putative *N- and C-terminal* PEST sequences that may regulate protein stability (1, 2, 14). The amino acid region Ser111-Ser131 is thought to represent another heteromeric interaction interface (3, 19, 20, 22) on D52, and the protein has been shown or predicted to bind to several proteins involved in endosomal function (Rab5 (20), Annexin VI (22), MAL2 (25)), ER to Golgi trafficking (PLP2, YIF1A (20)), Golgi structure (GOLGA5 (20)), intracellular lipid droplets (PLIN2 (10)), and the DNA double strand break repair enzyme (ATM (6)).

### D52 expression

*D52* transcripts are ubiquitously expressed, however protein expression is highest in epithelial cells that contain large secretory granules, most notably in the digestive system (8, 9). D52 expression is highest in the pancreas, mucosa of the stomach (chief and mucus secreting cells but absent in parietal cells), small and large intestine (goblet and Paneth cells), lung, testis, spleen, salivary, and lacrimal glands (8, 17). Additionally, high D52 expression is found in a number of cancers, including breast, colon, prostate, and ovarian cancer (7).

## 2. Specific function of D52 in the pancreas

D52 is a cytosolic and peripheral membrane protein that appears in a highly punctate pattern in the supranuclear and apical cytoplasm of acinar cells where it colocalizes with early, late, and recycling endosomes, and the Golgi apparatus but is not found on ER, secretory granules, or nuclei (9, 10, 13, 16, 24). Upon cell stimulation and elevation of intracellular calcium, D52 rapidly accumulates at the apical membrane and on endosomal compartments (**Fig 2**). A secretory role for D52 was first reported using D52-depleted permeabilized acinar cells where introduction of recombinant D52 enhanced amylase secretion by 2-3 fold, rescuing it to physiological levels (23). When ectopically expressed in CHO cells, D52 regulated endolysosomal secretion and cytokinesis in a phosphorylation dependent manner (21). Using adenoviral expression of D52 in cultured acinar cells, which are rapidly depleted

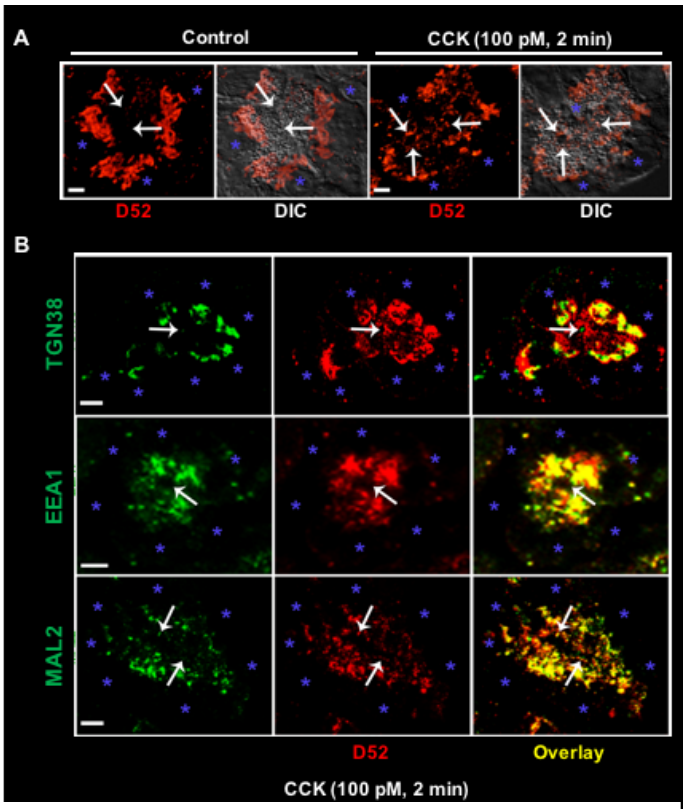


**Figure 2: D52 localizes to sub-apical *trans*-Golgi and endosomal compartments.** **A**: Brightfield immunofluorescence shows D52 accumulation in supranuclear regions under basal conditions and apical regions following 2 min CCK stimulation. **B**: D52 co-localizes with markers of the *trans*-Golgi (TGN-38) under basal conditions but expands to apical membrane, early endosome (EEA1), and sub-apical endosomal compartments (MAL2) upon CCK stimulation. Note all cells in B are CCK stimulated. Arrows denote the plasma membrane, asterisks denote nuclei, scale bars are 7 μm. This figure was adapted from (16)

of D52 expression, established that it regulates the constitutive-like (CLP) and minor regulated secretory pathways (MRP) in a phosphorylation-dependent manner (16). More recently, D52 and the CLP/MRP were found to be essential for VAMP 8-, but not VAMP 2-mediated zymogen granule secretion (13). Additionally, during acute pancreatitis, D52 and other associated CLP/MRP regulatory proteins are rapidly depleted in acini leading to a loss of VAMP 8-mediated secretion and intracellular proteolytic zymogen activation (14). Moreover, maintaining the D52-mediated apical endosomal trafficking pathway by D52 overexpression prevents trypsin activation during CCK-induced pancreatitis induction (13-15).

With regard to cancer, D52 has been shown to promote NIH3T3 cell transformation and enhance cancer invasiveness (12). Other studies have reported D52 is a negative regulator of the ataxia telangiectasia mutated kinase (ATM) which is a master regulator of the DNA damage response (6). A recent study demonstrated D52 is associated with a subset of lipid droplets in cancer cells suggesting it may play a role in lipid metabolism (10).

## 3. Tools for the study of this molecule

**a. Antibodies**

There are optimized antibodies publicly available (abcam #ab182578) and the Groblewski lab has produced an affinity purified polyclonal antibody (9).

**b. Primers**

Primers for qPCR have been validated for mouse: (F:TGCTGAAGACAGAGCCGG, R:ACGTCTTGCCACCCTTTG), and rat: (F:GCCATCACCTGGCATGGATT, R:CGCTCGGAGAGAGGTAGAGA).

**c. Vectors**

Adenoviral vectors expressing human HA-D52 wild type and mutants (S136E, S136A) have been produced by the Groblewski lab (16).

**d. Transgenic Mice**

Tamoxifen inducible *D52* floxed mice were produced by the UC Davis Mouse Biology Program for the Groblewski lab and are currently being characterized.

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