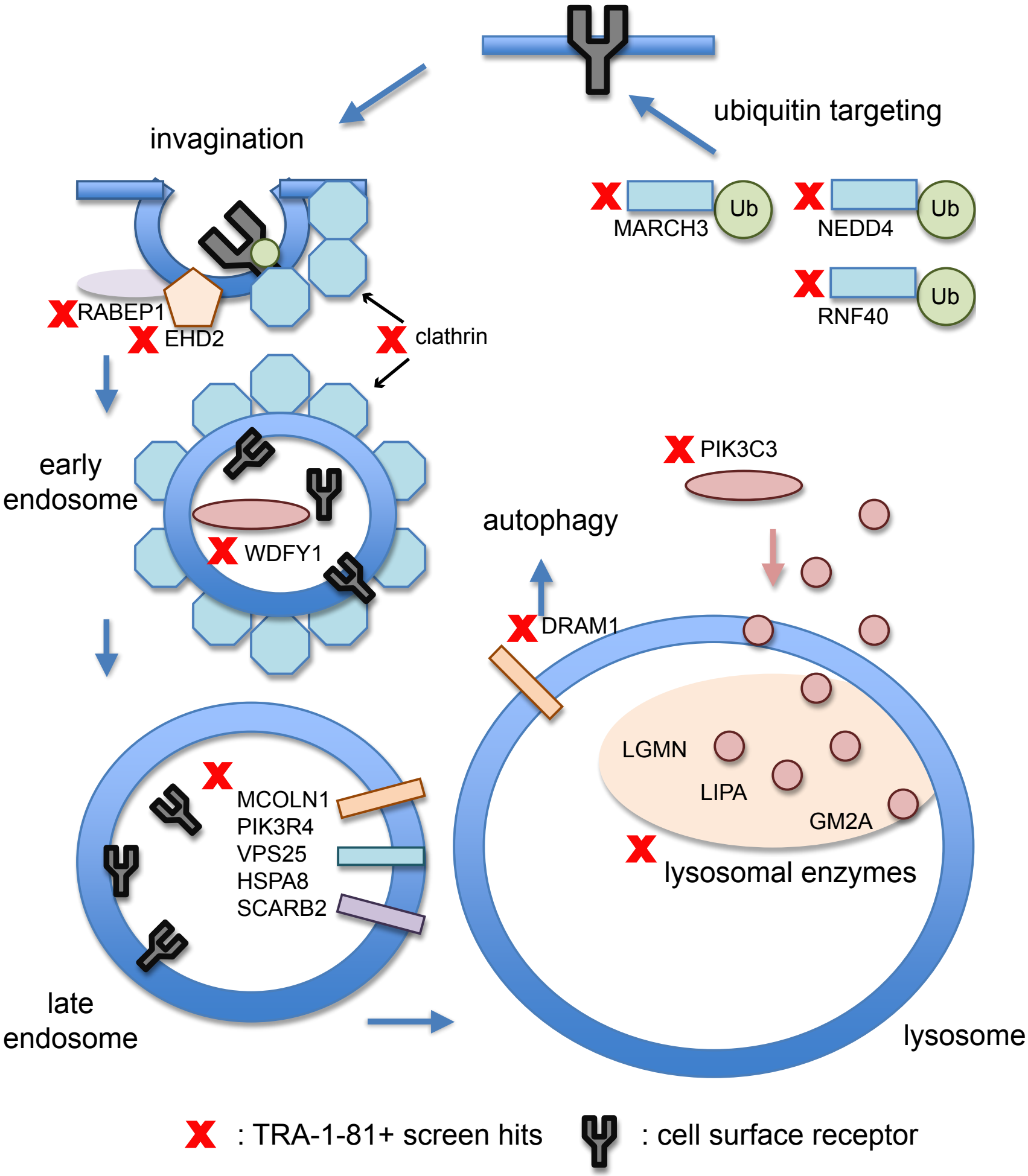
ubiquitin targeting
invagination
MARCH3
Ub
NEDD4
Ub
RNF40
Ub
RABEP1
EHD2
clathrin
early
endosome
WDFY1
PIK3C3
autophagy
DRAM1
LGMN
LIPA
GM2A
lysosomal enzymes
MCOLN1
PIK3R4
VPS25
HSPA8
SCARB2
late
endosome
lysosome
: TRA-1-81+ screen hits
: cell surface receptor

Figure 4 : Endocytosis and receptor mediated endocytosis are barriers to reprogramming

Using a combination of gene interaction network analysis and gene ontology analysis we identified genes central to endocytosis and receptor mediated endocytosis among the top 5% significance level (p=0.0502) TRA-1-81+ screen hits. Receptor mediated endocytosis frequently requires ubiquitination of the receptor and/or proximal surface proteins to target them for degradation. Screen hits MARCH3 (p=0.0152), RNF40 (p=0.0044), and NEDD4 (p=0.0172) have all been shown to mediate receptor ubiquitination and subsequent targeting for endocytosis [1-5]. Once targeted for proteolysis, transport vesicle formation requires RABEP1 (p=0.0262) and EHD2 (p=0.0262) which are screen hits. Early endosome formation involves formation of coated pits comprised of Clathrin (CLTA p=0.0046) as well as WDFY1 (p=0.0222), both top screen hits. Multiple screen hits were identified as vesicle surface proteins mediating late endosome-lysosome fusion (MCOLN1 p=0.0108, PIK3R4 p=0.023, VPS25 p=0.015, HSPA8 p=0.0242, SCARB2 p=0.014). Lysosomal lipases and proteases such as LIPA (p=0.0394), GM2A (p=0.035), and LGMN (p=0.0006) were screen hits as well as PIK3C3 (p=0.0166) which mediates lysosomal protein shuttling into the lysosome. Lastly, the lysosomal protein SLC17A5 (p=0.0192) and DRAM1 (p=0.044), a lysosome surface membrane protein associated with autophagy, were also screen hits.

1. Fukuda, H., Nakamura, N., & Hirose, S. (2006). MARCH-III Is a novel component of endosomes with properties similar to those of MARCH-II. *Journal of biochemistry*, *139*(1), 137–45. doi:10.1093/jb/mvj012

2. Jääskeläinen, T., Makkonen, H., Visakorpi, T., Kim, J., Roeder, R. G., & Palvimo, J. J. (2012). Histone H2B ubiquitin ligases RNF20 and RNF40 in androgen signaling and prostate cancer cell growth. *Molecular and cellular endocrinology*, *350*(1), 87–98. doi:10.1016/j.mce.2011.11.025

3. Anan, T., Nagata, Y., Koga, H., Honda, Y., Yabuki, N., Miyamoto, C., Kuwano, a, et al. (1998). Human ubiquitin-protein ligase Nedd4: expression, subcellular localization and selective interaction with ubiquitin-conjugating enzymes. *Genes to cells : devoted to molecular & cellular mechanisms*, *3*(11), 751–63. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9990509

4. Wang, X., Shi, Y., Wang, J., Huang, G., & Jiang, X. (2008). Crucial role of the C-terminus of PTEN in antagonizing NEDD4-1-mediated PTEN ubiquitination and degradation. *The Biochemical journal*, *414*(2), 221–9. doi:10.1042/BJ20080674

5. Persaud, A., Alberts, P., Amsen, E. M., Xiong, X., Wasmuth, J., Saadon, Z., Fladd, C., et al. (2009). Comparison of substrate specificity of the ubiquitin

ligases Nedd4 and Nedd4-2 using proteome arrays. *Molecular systems biology*, *5*(333), 333. doi:10.1038/msb.2009.85