protein, the average viral target was distinct in four ways: it was significantly more connected to other proteins; it was in a more central network position; it participated in more cellular pathways; and it was more likely to be engaged in central positions within these pathways (Fig. 1b and Supplementary Fig. 6d, e). These properties are consistent with a strong influence on pathways and effective control of biological networks[21], which is in line with the parsimonious use of viral genetic material, and coevolution of the virus with the host organism.

Our large host-factor survey using a defined cellular set-up offers the unique opportunity to identify host-cell perturbation strategies pursued by individual viruses, families and groups. On the basis of the humPPI, 70% of the viORF-interacting cellular factors formed a coherent protein–protein interaction network (Supplementary Fig. 7a). When mapped on the entire humPPI, viral targets seemed to occupy central positions (Supplementary Fig. 7b). We also grouped the cellular targets on the basis of their interaction with viORFs from single-stranded (ss) or double-stranded (ds) RNA or DNA viruses and found that about half of the viORF targets linked to a single viral group, and the rest interacted with viruses of more than one group (Fig. 2a). Statistically significant enrichment for individual gene ontology (GO) terms, representing categories of biological processes, could be identified for each subnetwork. Proteins targeted by ssRNA(−) viORFs were enriched for processes related to protection of the viral genome and transcripts from degradation or detection by the host, and for those promoting efficient viral RNA processing (Fig. 2a). This is illustrated by the interaction between NS1 of influenza A virus (FluAV) with the 5′→3′ exoribonuclease XRN2, and among the NSs protein of Rift Valley fever virus, the mRNA export factor RAE1 and the nuclear pore complex protein NUP98. In contrast, dsRNA virus targets were enriched for protein catabolic processes (Fig. 2a) with both rotaviruses and reoviruses (NSP1 and σ3) engaging SKP1–CUL1–F-box protein complexes (containing FBXW11, Cullin-3, and Cullin-7 and Cullin-9, respectively), which mediate protein degradation.

To determine which cellular signalling pathways are targeted by viORFs and to look for differences between DNA and RNA viruses, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) annotations (Supplementary Table 4). Clear distinctions in preferences were observed between the different viral groups, with viORFs of RNA viruses targeting the JAK–STAT and chemokine signalling pathways, as well as pathways associated with intracellular parasitism, and viORFs of DNA viruses targeting cancer pathways (glioma, acute myeloid leukaemia and prostate cancer) (Supplementary Table 4). Among the viral targets that are involved in multiple cellular pathways were two catalytic and three regulatory subunits of the phosphatidylinositol-3-OH kinase family, identified with the FluAV NS1 protein and with the TLR inhibitory protein A52 of vaccinia virus (VACV) (Supplementary Fig. 8a)[4]. We functionally validated these interactions and identified a critical role for one of the catalytic subunits (PIK3CA) in TRIF-mediated IFN-β promoter activation (Supplementary Fig. 8b–d).

The higher probability of viORFs targeting cellular proteins that link different pathways (Fig. 1b and Supplementary Fig. 6d) prompted us to map which of these pathway connections were preferentially targeted and thus were probably disrupted (Fig. 2b), and to compare the disruption patterns brought about by viORFs from DNA viruses with

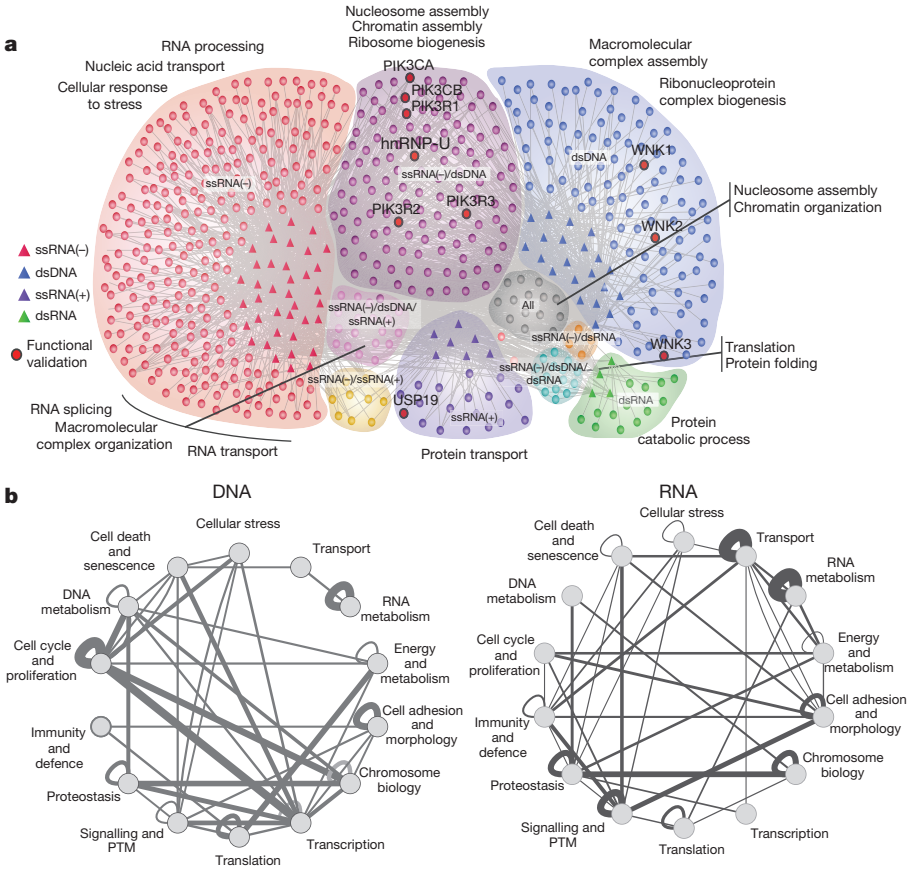

Figure 2 | Network of identified targets and network perturbation induced by viORFs. **a**, Network representation of all the viORF–target-protein interactions with viral targets grouped according to the genome type of the interacting viORF(s). Proteins identified in the negative control cell line were subtracted as non-specific binders. Triangles represent viORFs; circles represent viral target proteins. Protein interactions functionally validated in detail in the study are marked in dark red. Up to three GO terms significantly enriched in the corresponding viral target subsets are shown around the network to highlight specific functions. **b**, viORFs targeting one or two proteins that physically interact and are involved in one or more biological processes have the potential to perturb communication or synchronization within or between the given process(es). Significant perturbations were determined ($P < 0.001$) using targets of viORFs derived from DNA or RNA viruses; edge thickness represents a normalized perturbation score.